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REVIEW

The evolutionary cancer genome theory and its reasoning

Vladimir F. Niculescu*

Kirschenweg 1, Diedorf, Bavaria, 86420, Germany

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ABSTRACT

Oncogenesis and the origin of cancer are still not fully understood despite the efforts of histologists, pathologists, and molecular geneticists to determine how cancer develops. Previous embryogenic and gene- and genome-based hypotheses have attempted to solve this enigma. Each of them has its kernel of truth, but a unifying, universally accepted theory is still missing. Fortunately, a unicellular cell system has been found in amoebozoans, which exhibits all the basic characteristics of the cancer life cycle and demonstrates that cancer is not a biological aberration but a consequence of molecular and cellular evolution. The impressive systemic similarities between the life cycle of Entamoeba and the life cycle of cancer demonstrate the deep homology of cancer to the amoebozoans, metazoans, and fungi ancestor that branched into the clades of Amoebozoa, Metazoa, and Fungi (AMF) and shows that the roots of oncogenesis and tumorigenesis lie in an ancient gene network, which is conserved in the genome of all metazoans and humans. This evolutionary gene network theory of cancer (evolutionary cancer genome theory) integrates previous findings and hypotheses and is one step further along the road to a universal cancer cell theory. It supports genetic cancer medicine and recommends soma-to-germ transitions—referred to as epithelial-to-mesenchymal transition in cancer—and cancer germline as potential targets. According to the evolutionary cancer genome theory, cancer exploits an ancient gene network module of premetazoan origin.

Introduction

In the past 2 decades, certain morphologic, physiological, and genetic similarities between cancer and protists have been observed, which suggest the ancient origin of cancer.[1-9] New disciplines such as evolutionary cell biology, genomic phylostratigraphy, and comparative life cycle analyses, addressing the transition to multicellularity and cancer, have confirmed this hypothesis.[7,10-15] In 2007 Erenpreisa and Craig introduced the term cancer life cycle as "a recapitulation of ancient protozoan life-cycle programs, which feature aspects of germ-line cells." They proposed "an intrinsic cancer life cycle consisting of a continuous sequence of mitotic and polyploid cell cycles."[16-19]

Comparative life cycle analyses of Entamoeba and cancer underscore the evolutionary deep homology of the 2 cell

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*Correspondence and requests for materials should be addressed to Vladimir F. Niculescu, Kirschenweg 1, Diedorf, Bavaria 86420, Germany. *Email address:* vladimir.niculescu@yahoo.com

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systems.[14] Among the overwhelming evidence for deep homologies with the common ancestor and with each other are the quite similar functions of their germ and soma (G+S) cell systems. These homologies involve (1) stemness and germline stem cell (GSC) generation, (2) germline sensitivity to evolutionary hyperoxia up to 6% O_2 content, (3) loss of stemness because of excessive oxygen and DNA damage, (4) genome repair structures for restoring stemness, (5) phenotype plasticity, and (6) switching from germ-to-soma and vice versa (Table 1).

But why are the life cycles of Entamoeba and human (metazoan) cancer so similar, while this parallelism is less known, if at all, in other amoebae? There are 3 clear reasons for this. First, Entamoebae are the most studied amoebae because of their pathogenicity, and a large number of culture media and studies have been performed over the years. Second, Entamoeba and cancer cells parasitize in similar oxygen-poor and oxygen-rich regions of the human body, and this feature has been accounted for in experiments. Third, the homologous germ and soma genomes of Entamoebae and cancer are exposed to the same environmental stimuli found in organs, tissues, and bloodstream that activate the amoebozoa, metazoa, and fungi (AMF) genes.

Both "sister life cycles" of cancer and Entamoeba provide insights into the life cycle of the common AMF ancestor and its unicellular AMF genome, which was inherited by the branching sister clades of AMF.[14] Unfortunately, the close relationship between cancer and the common ancestor was overlooked for a long time. Many authors and theories have addressed the question of how cancer arises and develops within the host organism, but the question of what cancer is, why it reactivates a complex gene module from the distant past, and where this complex gene network comes from has received much less attention.

According to Anatskaya et al,[13] several researchers refer to cancer as an "atavistic shift associated with polyploidy and driven by epigenetic changes." They reactivate bivalent developmental genes. They also suggested that artificially induced or derailed polyploidization or incomplete tetraploidy "may be responsible for carcinogenesis and may be an important contributor to epigenetic changes." Quite a few researchers speak of an "atavistic cancer theory," which would be confirmed by phylostratigraphic analyses.

It is this author's opinion that the term "atavistic" is not appropriate in the case of oncogenesis. Atavistic means a sporadic "very old habit from a time long past" as defined in the Cambridge Advanced Learner's Dictionary & Thesaurus. Günter P. Wagner defines an atavism as "a morphophysiological trait controlled by an ancient gene regulatory network (GRN) that survives periods of disuse and can be reactivated and reused in later lineages, even if it was not used in the immediate ancestors."[21] Cancer is therefore not an atavism, because it has been continuously expressed by all intermediate ancestors of humans, mammalians, and vertebrates over the past 800 million years. It occurs in about half of each population and each generation.

Abbreviations

ACD – asymmetric cell division
aCLS – alternative cyst-like structure
AMF – amoebozoa, metazoa, and fungi
CSC – cancer stem cell
D1 cell – self-renewing daughter cell
D2 cell – cell committed for stem cell formation
DDR – DNA damage response (DNA damage repair)
DNA DSB – double-strand break
EM – early metazoan
EMT – epithelial-to-mesenchymal transition
G+S – germ and soma
GSC – germline stem cell
GST – germ-to-soma transition
HRD – homologous recombination deficiency
HRR – homologous recombination repair
ICD – induced cyst development
MC – multicellular
MET – mesenchymal-to-epithelial transition
MGRS – multinucleate genome reconstruction syncytia
Mya – million years ago
OCB – oxygen-consuming bacteria
PGCC – polyploid giant cancer cell
RGP/GSC – reproductive polyploid cycle forming germline stem cell
RP – reproductive polyploidization
SCD – symmetric cell division
SGT – soma-to-germ transition
UC – unicellular
WGD – whole-genome duplication

There is also much confusion regarding cancer polyploidy. Many authors use knowledge of somatic tissue polyploidy to understand polyploidization in cancer.[13] However, cancer polyploidy is not somatic polyploidy.[14] Cancer polyploidy is reproductive germline polyploidy. It gives rise to nascent cancer stem cells (CSCs) via autonomous cyst-like structures (aCLSs), also termed native polyploid giant cancer cells (PGCCs). Stress leads to loss of stemness, and stemness recovery occurs exclusively via repair PGCCs. These giant cancer cells repair severe DNA defects and reorganize the germline genome through the same repair mechanisms as multinucleate genome reconstruction syncytia (MGRS) in amoebae.

In the human genome, there are several suppressed gene modules from the deep past, and they are in a state of facultative or partial reactivation. As reported by Aydan Burn, "human endogenous retroviruses (HERV), which make up around 8% of the human genome, were left behind as a result of infections that primate ancestors suffered millions of years ago. They are part of the human genome due to how they replicate. About 60,000 proviruses in the human genome demonstrate the long history of the many pandemics during the evolution of the primate genome. Some of these genetic remnants within the human genome still retain the ability to make viral proteins. Particularly interesting HERV genes are active in tumors, and infections

Table 1 The life cycle of amoebozoans and cancer; homologies and differences

Life Cycle	Amoebozoa	Cancer
1. Germline (non-gametogenic)		
SCs producing cell line	✓	✓
Generates SCs in conditions of normoxia	GSCs	CSCs (native CSCs)
Performs RP/SC cycles	✓	✓
Normoxic ACD (HRR) phenotype	✓	✓
ACD promoting protein	Unknown	NUMB[20]
Low-oxygen phenotype (<6.0% O_2 content)	✓	✓
Repairs DNA defects by HRR	✓	✓
Proliferates by ACD	✓	✓
Forms 2 nonidentical daughter cells	✓	✓
(Self-renewing ACD/d1 and differentiating ACD/d2)	✓	✓
ACD/d2 cells differentiate polyploid reproductive structures	Reproductive cysts	aCLSs (native PGCCs)
Produces SC via RP/SC cycles and haploidization	✓	✓
Number of SCs per cycle	$N = 16$	Unknown
Defective SCD (HRD) phenotype	✓	✓
Hyperoxic phenotype (>6.0% O_2 content)	✓	✓
Proliferates by symmetric cell division (SCD)	✓	✓
Forms identical daughter cells	✓	✓
DNA defects and genome damage due to excess oxygen	✓	✓
HRD	✓	✓
Loss of function: loss of stemness and GSC, no GSCs	✓	✓
Fusionable progeny as observed in	Hyperoxic cultures	Glioblastoma cell cultures
Germline restoration	✓	✓
Occurs by soma-to-germ transition	SGT + encystment	EMT
Forms new "healthy" germline clones	✓	✓
Forms secondary stem cells	GSCs	Secondary CSCs
Increased virulence and pathogenicity	✓	✓
Germline genome repair	✓	✓
Stressor	Oxygen excess	Oxygen excess, therapy
Fusionable damaged germline cells form syncytia:	MGRS	PGCC
Polyploid by nuclear fusion	✓	✓
Damaged RP/GSC cycles	GST	Secondary CSCs
Nuclear fusion to, polyploid giant nuclei,	✓	✓
DNA debris excision, reductive nuclear division	✓	✓
Sporulation (buds) and	✓	✓
MGRS/PGCC whitout cell fusion	Not given	✓
2. Somatic cell line	✓	✓
Hyperoxia resistant	✓	✓
Unexpressed stemness	✓	✓
Protects germline genome	✓	✓
Cell line conversion	SGT	EMT

ACD, asymmetric cell division; *CSC*, cancer stem cell; *CLS*, cyst-like structure; *EMT*, epithelial-to-mesenchymal transition; *GSC*, germline stem cell; *GST*, germ-to-soma transition; *HRD*, homologous recombination deficiency; *HRR*, homologous recombination repair; *MGRS*, multinucleate genome reconstruction syncytia; *PGCC*, polyploid giant cancer cell; *RP*, reproductive polyploid cycle; *SC*, stem cell; *SCD*, symmetric cell division; *SGT*, soma-to-germ transition.

as well as during embryonic development."[22] A most recent research field explores simultaneously host and pathogen genomes, through so-called genome-to-genome studies. This new research area promises to be particularly effective and could help to better understand the interrelation between the conserved premetazoic gene network and the metazoic host genome.

It is unclear how the evolution of premetazoans proceeded toward multicellularity and whether this was more of an exogenous or endogenous origin. In other words, the following questions can be asked: (1) did twin cells with the identical AMF genome but different developmental fate drive evolution toward multicellularity? or (2) did multicellular (MC) evolution start from a single AMF genome that shaped the metazoan genome by adding new genes, functions, and regulatory networks? Conventional wisdom rather favors the first variant, which may have arisen in connection with primitive cell colonies of the transition era. The evolution of the sponges argues more for the second endogenous variant.

From the presented information, it follows that in the transition period, many evolutionary attempts were made with transformed unicellular (UC) genes or newly founded genes, but they usually resulted in genetic instability and dead ends. In such cases, the stable UC G+S system had to be reactivated for survival and the saved cells gained time to

start a new evolutionary attempt. Genes of failed attempts were retained and silenced. In this way, many new genes were added to the premetazoan genome. They enriched the genomes of the transition period.

According to this author, the constant back and forth and the resulting advantage were so essential for evolution that neither transit organisms nor unstable early metazoans (EMs) could survive without it. They retained the G+S life cycle variant as a kind of passive-facultative "ultima ratio" and passed its genes and gene regulatory network onto all metazoan lineages yet to come. The G+S life system was not permanently shut down but only "temporarily" suppressed. Antisuppressor pathways for reactivating the G+S life cycle were also retained. The ancient suppressor and antisuppressor genes are the archetypes of tumor suppressor genes and oncogenes.

Thus, when a metazoan cell is metabolically threatened or genomically deficient, irreversibly loses vital functions, and has no means of repair, it invokes the old survival pathway and reactivates the AMF ancestral G+S system. In more evolved metazoans, the reactivation is cancerous and proves to be an aggressor against the host organism. This antihost ability also has evolutionary origins: the cell returning to the primitive G+S life cycle (sentinel cell) was able to eradicate the remaining failed colony cells.

Recent findings on Entamoeba germline provides valuable insights into the lesser-known cancer germline and the production of CSCs and provides more clarity on the intrinsic pressure that forces the permanent production of "healthy" germlines clones and CSCs.[23] After branching, amoebozoans and metazoans evolved differently. The G+S cell system of Entamoeba retained the ancestral traits of the UC AMF ancestor, including encystation. In cancer, the G+S cell system evolves against the host organism and acquires numerous additional antihost genes. It dispenses with unnecessary UC structures, such as the cyst wall, and evolves much simpler structures, such as the thin envelope of PGCCs. However, it retains the reproductive polyploid cycle that gives rise to nascent CSCs (RP/CSC cycle).

Meanwhile, molecular biology studies have shown that cancer accesses the UC gene module embedded in the human genome, which contains historical polyploidy genes.[7,13,21-30] These genes were laid down in the common ancestor of AMF clades about 900 million years ago (Mya) ago and passed to EMs around 700 to 600 Mya. The evolutionary history of polyploidy genes and cancer polyploidy is still not clearly understood. Therefore, it is important not only to discuss the evolutionary roots of polyploidy and polyploidy genes but also to make a comparative analysis between the germline polyploidy of the AMF ancestor and common somatic polyploidy of metazoans and human.[24] In cancer, it has long been overlooked that nascent primary stem cells (CSCs) are produced by the germline and must be considered GSCs.[31] Moreover, there is recurrent confusion between germline and CSC fractions, and many of the germline features such as self-renewal and asymmetric cell cycling have been attributed exclusively to stem cell fractions.[32] It has not become clear that reproductive polyploid RP/GSC cycles and CSC production occur only in the ACD phenotype of normoxic germlines (low-oxygen germline phenotype) capable of asymmetric cell division (ACD).

The present work goes back to the AMF germline and its reproductive polyploidy and the loss of stemness function because of stress and hyperoxia. Differences between germline polyploidy and somatic are also highlighted. Furthermore, this work describes the mechanisms of genome protection, germline replacement, and stemness restoration. The term normoxia refers to the times when the G+S life cycle of the AMF ancestor evolved. They correspond to the low-oxygen period between 900 Mya and 600 Mya when oxygen levels were about 4% (20% partial pressure of atmospheric oxygen level).[33] Such normoxic low-oxygen zones occurring in the intestine and stem cell niches are the optimal environment for germline activities. When leaving normoxic zones, germline cells are confronted with excess oxygen content that damages their genome (loss of stemness). However, the germline can rescue its genome by germ-to-soma transition (GST, mesenchymal-to-epithelial transition [MET]-like) and soma-to-germ transitions (SGT, epithelial-to-mesenchymal transition [EMT]-like) processes. Through this adaptation, the original G+S system gained the ability to survive in both low-oxygen niches and more oxygenated environments and tissues.[34]

The Basic G+S System of Cancer and Amoebae Originates From the Common AMF Ancestor

Most of the knowledge that we have about the AMF ancestor and its G+S cell system comes from recent insights into the complex life cycle of parasitic amoebae. However, this was not always the case. Until about 2020, parasitologists considered Entamoeba to have a simple life cycle, exclusively consisting of 2 stages: a vegetative trophozoite and a dormant cyst.[35] This is a highly simplified and inaccurate assertion that defies evidence. In addition, the statements that the cyst is a case of (terminal) differentiation[36] or stage conversion and "minuta" is merely a small precyst stage that cannot proliferate are not valid.[37] The truth is that the life cycle of Entamoeba remained virtually unknown for a long time.

This is because Entamoebae has historically been cultured at oxygen levels above 5.7% O_2 (free of bacteria)[38] without considering that the primary habitat of parasitic amoebae is the gut with low oxygen levels below 5.7% O_2 (amoebic normoxia). Unfortunately, axenic cultures without bacteria have prevented the use of physiological culture methods and led to the fatal misconception that Entamoeba populations would be homogeneous consisting of identical trophozoites. Only a few researchers were able to prove that vegetative cyst-free populations are heterogeneous and more phenotypes exist.[39-41]

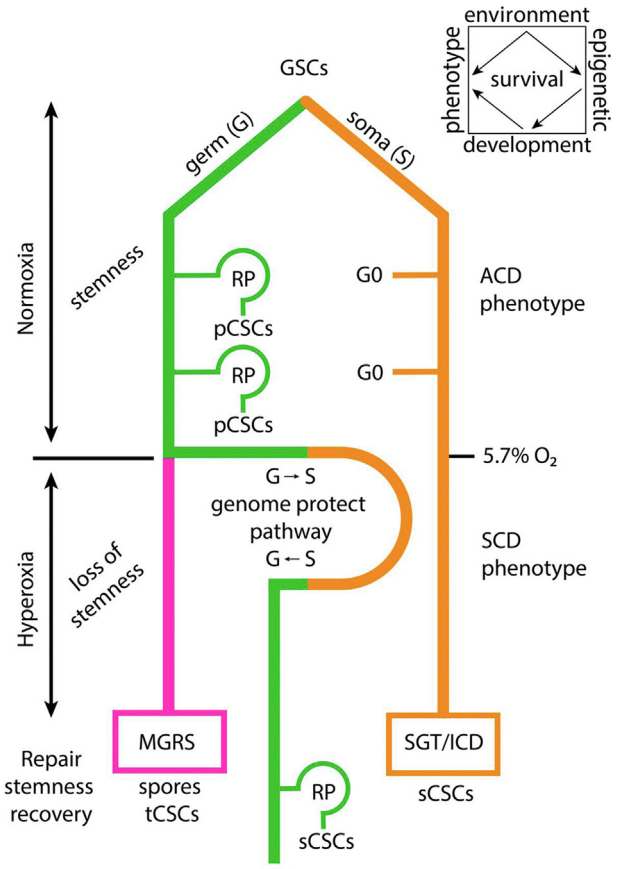

Figure 1 Genome protection by MET-EMT (GST-SGT) cycles. The G+S life cycle begins with a germline stem cell (GSC) that differentiates into an oxygen-sensitive germline (green) and an oxygen-resistant soma line (yellow) under various low oxygenic conditions. Cell line differentiation and life cycle development are oxygen dependent: (i) Under normoxic G+S conditions (5.7-6.0% O_2 content), both cell lines proliferate by asymmetric cell division (ACD) with the difference that only germline's ACD/d2 phenotype can perform RP/GSC cycles and form CSCs (primary pCSCs or nascent CSCs), while the ACD/d2 phenotype of the somatic cell line remains in a reversible G0 state; (ii) tissue with more than 5.7-6.0% O_2 (germline hyperoxia) forces all cells into symmetric cell division and damage the oxygen-sensitive germline. Only the somatic SCD phenotype maintains genomic integrity, while the oxygen-sensitive germline loses it. Characteristics of the hyperoxic defective germline phenotype (red) include loss of function (no stem cell production), irreparable DNA DSB, and HR deficiency (HRD phenotype) but also abnormal hyper-polyploid cell cycles with supernumerary centrosomes, tripolar mitotic spindles as well as multinuclearity. The defective germline cells cannot be reintegrated in the G+S life cycle, serving only for genomic reconstruction by MGRS/PGCC mechanisms and sporogenesis. The hyperoxic somatic cell fraction protects the genome against oxygen, maintains its integrity, and forms new germline clones. Normoxic GST (MET) and SGT (EMT) processes originating from hyperoxic somatic cells prevent genome alteration. pCSCs, sCSCs, tCSCs, primary, secondary and tertiary CSCs. Top right: Burggreen's concept of developmental phenotype switching demonstrates the interactions between phenotype, environment, epigenetic changes, and development, that lead to the successful survival of the cell system in stressful environments.[42] CSC, cancer stem cell; DSB, DNA double-strand break; G+S, germ and soma; GST, germ-to-stoma transition; MGRS, multinucleate genome reconstruction syncytia; pCSC, primary (nascent) CSC; PGCC, polyploid giant cancer cell; RP, reproductive polyploid cycle; SCD, symmetric cell division; sCSC, SGT, soma-to-germ transition; secondary CSCs formed through SGT (EMT) processes; tCSC, tertiary CSC formed through MGRS (PGCC) repair.

In truth, parasitic amoebae have a complex germ and soma life cycle (Figure 1). It consists of an oxygen-sensitive germline that can form reproductive cysts and stem cells and an oxygen-resistant soma line that cannot.[23,43] In normoxic oxygen-consuming bacteria (OCB) cultures, cysts are formed by the germline via asymmetric cell cycles. Hyperoxia above 5.7% impairs ACD and forces the germline to divide symmetrically without cyst and stem cell formation.[44] Both ACD and SCD germline phenotypes (low-oxygen and high-oxygen phenotypes) could be obtained in OCB cultures, and both germ and somalines can cycle back and forth through GST and MET-like and SGT and EMT-like. Thus, GST and SGT processes (MET- and EMT-like processes) are intrinsic features of the G+S cycle. This is a striking similarity to the life cycle of cancer.

Growth under normoxic conditions helps to correct previous misconceptions about the Entamoeba life cycle and the homology to cancer.[44] Normoxic OCB cultures, subcultures, and passages provide all the stimuli that germ and soma cells need to complete the G+S cycle. OCB stimuli induce RP/GSC cycles, stem cell formation, and differentiation of new germline clones (Figure 1).

Understanding the G+S life cycle of parasitic amoebae was the result of serendipitous circumstances encountered by the author during his many years of research in this field. It begins with the introduction of normoxic culture conditions for *Entamoeba invadens* and the realization that the life cycle of parasitic amoebae under normoxic conditions is much more complex than previously thought.[23,44] *E invadens* was grown in sediments with metabolically suppressed OCBs. The growth in OCB cultures reveals that Entamoeba's G+S cell system consists of a germline (*Minuta*) that can form cysts and a soma line (*Magna*) that does not. Cysts were formed at the border between normoxia and hyperoxia. Similar G+S systems have been reported in various organisms such as Tetrahymena, but also in Giardia or the facultative parasite *Colpoda cucullus*.[45-47] Homologies with the life cycle of Entamoeba were also observed in invertebrates such as sponges and Drosophila.[48,49]

How Germline and Soma Cell Line Evolved and Derived From Each Other

The evolution of the AMF ancestor and its life cycle was closely related to environmental stress and changes in oxygen levels. Two evolutionary steps led the AMF precursor to its sophisticated life cycle. The first was the formation of

reproductive cysts and stemness in spatiotemporal low-oxygen periods and the second was the formation of a second oxygen-tolerant soma cell line in times of increased oxygen content.

From the simple protective cyst to reproductive cysts, germline, and stemness

The formation of protective cysts in primitive protozoa began as early as 1000 Mya ago in response to harmful living conditions. Primitive vegetative cells learned to protect themselves against stress by forming a thickened cell wall of chitin and other glycopolymers, leaving the protective chitin shell when better times came.[50-53]

As observed in Entamoeba, cyst formation begins from a state of G1 similar to the G1/G0 differentiation in mammals and neoplasia.[54] S-phase and G2/M cells do not respond to encystation stimuli and stem cell formation; they enter cytokinesis and form 2 identical juvenile G1 cells; both are capable of cyst formation. Cell cycle progression normally precludes encystation. However, because the G1/S barrier checkpoint was not finally formed in earlier evolutionary times, replication and cyst wall formation overlapped and were not mutually exclusive. This could lead to the formation of a polyploid nucleus with 2 complete genome copies. Because of a lack of space within the cyst, depolyploidization occurred by reductive nuclear division (amitosis). This alternative could later lead to several rounds of intracystic replication that form multiple daughter cells through amitosis. In times of low resources and harmful environmental conditions, the multitude of offspring was a clear survival advantage.

There are no data on this AMF period, which occurred about 1000 Mya ago, and neither paleontological nor geological evidence is available.[14] The AMF evolution can only be understood through evolutionary protistology and analytical comparisons among amoebozoans, EMs, and cancer cell systems.[14,15] The scientific literature for this period is sparse to nonexistent, but it is assumed that polyploid cysts, once established, have evolved a gene network and developmental program for stemness and differentiation to thus transform the former primitive vegetative cell line into a true germline.

O_2-sensitive germline and GSCs

Early in evolution, there was a substantial increase in environmental oxygen levels that damaged the oxygen-sensitive germline cells and impaired their ability to polyploidize and form cysts and stem cells. The loss of ACD and stemness potential under conditions of elevated oxygen conditions were inherited from the ancestor to the germlines of amoebozoans, metazoans, and cancer. The homology among the different stem cell types such as GSCs of amoebozoans and embryonic stem cells, adult/somatic stem cells, and CSCs goes back to the AMF ancestor: all stem cell types have an AMF origin and use the genes of the gene network conserved in human and mammalians.[55]

Similar to Entamoeba stem cells, mammalian and human stem cells undergo several adverse changes when cultured at higher oxygen concentrations than those present in the niche microenvironment. Hyperoxia leads to oxidative stress and metabolic turnover. It reduces proliferation and motility, altered self-renewal, stemness, and differentiation by loss of function. As noted by Mas-Bargues et al,[56] "all of these consequences can be avoided if stem cells are cultured at their physiological oxygen levels." Excess oxygen causes "changes in the expression of genes involved in DNA damage response (DDR) pathways, such as the MLH1, RAD51, BRCA1, and Ku80, causing changes in TP53 (p53) genes, and loss of genomic integrity."[57-61] It shows that all stem cells, whether from amoebozoans, metazoans, or cancer, are subject to the same low-oxygen control rules.

The AMF period was the time of life cycle diversification and division of labor. First, the old O_2-sensitive cell line retained its polyploid reproductive function as long as it resided in a normoxic environment with less than 6.0% O_2 content (Figure 2). It took over the function of a germline capable to produce GSCs (nascent GSCs). Nascent GSCs are produced only from the normoxic ACD phenotype, which proliferates by ACDs. Second, the new O_2-resistant cell line acquired the function of a cyst-free somatic cell line. It proliferates well both in normoxic and hyperoxic living conditions without damage. Thus, unlike the germline, the somatic cell line can protect the genome from excess oxygen. Moreover, somatic cells can form new healthy germline clones and replace damaged germlines and GSCs by SGT. The G+S cell system developed by the AMF ancestor has enormous regenerative power and survival potential. This is the biggest immortality problem in fighting cancer. Another major problem in cancer is the difficulty to distinguish different cell stages from each other. Stem cells are often confused with germline cells, and many of the properties of the germline are attributed to stem cells. Specific cell status markers can help to resolve this problem.[32,62]

Germline ploidy and reproductive RP/GSC cycles

Polyploidy in cancer and amoebae is reproductive germline ploidy.[31,63] It differs fundamentally from somatic polyploidy observed in human and mammalian tissues (myocardium, bone marrow, liver, placenta, and tissue regeneration) or genetic polyploidy. Germline polyploidy has the highly significant function of producing stem cells (GSCs and CSCs). However, only the "healthy" germline cells of the ACD phenotype with ACD can form stem cells. Damaged germline cells of the SCD phenotype with symmetric cell division (SCD) cannot form stem cells and undergo abnormal hyperploid cell cycles resulting from defective mitoses (nondevelopmental polyploidy).[64]

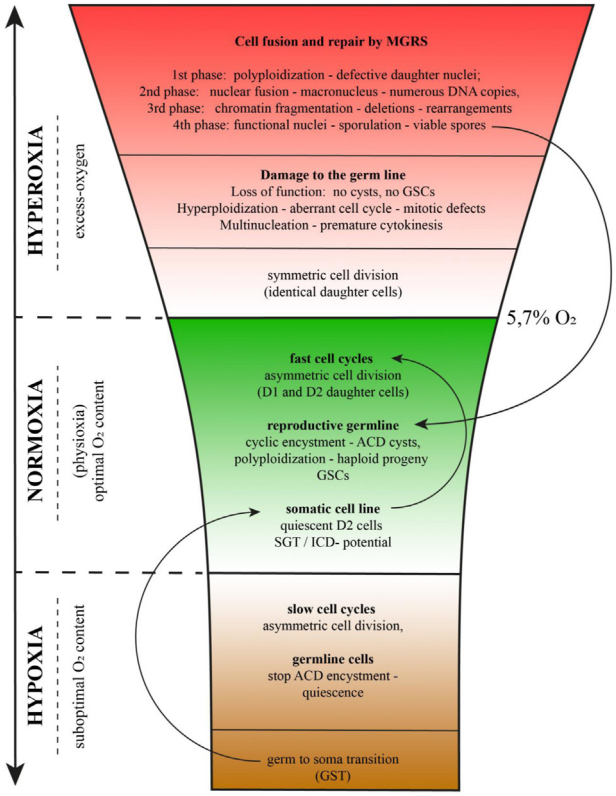

Figure 2 The evolutionary oxygen requirements of the ancestral G+S life cycle as observed in Entamoeba. The normoxic region contains up to 5.7% O_2, hyperoxic region more than 5.7% O_2; a more intense color means more oxygen. In Entamoeba, the intestinal oxygen gradient with 0.1% to 5.7% O_2 content is the physioxic environment of the germline that has normoxic (green) and hypoxic (red) zones. The normoxic zone allows the expression of all germline functions. The hypoxic zone (brown) decreases and stops germline functions without damaging the germline. Hyperoxia disrupts the ACD phenotype and damages the germline. Damaged germline cells proliferate like SCD cells by symmetric cell division; however, they show several mitotic defects, such as hyperploidization, multinucleation, multipolar divisions with supernumerary centrosomes, and tripolar mitotic spindles. Several genes controlling the cell cycle are downregulated. The G+S cycle has mechanisms to generate new "healthy" germlines from somatic cells through SGT events or from damaged germline cells through the MGRS repair pathway. ACD, asymmetric cell division; G+S, germ and soma; GSC, germline stem cell; GST, germ-to-soma transition; ICD, induced cyst development; MGRS, multinucleate genome reconstruction syncytia; SCD, symmetric cell division; SGT, soma-to-germ transition.

Lazzeri et al[65] pointed out, "somatic polyploidy observed in human and mammalian tissue is part of the normal postnatal morphogenetic program (organogenesis) but can also occur in response to stress and pathological stimuli. In adult organs such as the heart and liver, polyploidization of cardiomyocytes and hepatocytes occurs by limiting cytokinesis and karyokinesis and slowing proliferation. In adult mammalian organs with low mitotic activity, polyploidization occurs as a result of hyperfunction and stress. Stress promotes polyploidization in both quiescent and proliferating cells."

Germline polyploidy is the reproductive polyploidy of the RP/GSC cycle and is performed by the committed ACD/d2 cell—the sister cell of the self-renewing ACD/d1 cell.[55] Germline polyploidy is restricted to "healthy" germlines. It differs from the somatic polyploidy observed in tissue (heart muscle, bone marrow, liver, and placenta) and tissue regeneration. Some stages of the RP/GSC cycle also occur in metazoans, usually covert and associated with reproductive structures. In contrast to the RP/GSC cycles performed by the healthy ACD germline to generate native stem cells (GSCs), PGCC cycles are pathways to repair severe DNA damage and remodel the damaged genome.[65-67] It is a repair pathway homologous to the MGRS repair pathway of amoebozoans. Cancer PGCC cycles repair the therapeutically damaged germline cells and germline cells with defective SCD phenotype, via or without cell fusion.[63] Both germline cells of SCD and therapeutically damaged germline cells lose their stemness expression and need to be repaired.

Soma cell line and phenotypic plasticity

Somatic cell lines also exist as distinct ACD and SCD cell phenotypes. Under normoxic conditions with less than 6.0% O_2, cells of the ACD/d1 phenotype proliferate by fast cycling, whereas the ACD/d2 phenotype does not undergo differentiation and does not produce stem cells, although it has stemness and differentiation potential. ACD/d2 cells remain in a state of G0 quiescence from where they return to the mitotic cell cycle. Under conditions of hyperoxia with more than 6.0% O_2 content, somatic ACD cells switch into the SCD phenotype and form identical daughter cells; they are oxygen resistant and retain stemness potential. Once they return to low-oxygen zones, SCD cells transit to the ACD phenotype, which is capable of RP/GSC cycles and stem cell formation. Soma is the vehicle for germline transmission.

The statement that soma is the carrier for germline transmission comes from the article by Dawkins.[68] This is consistent with the data from Figure 1 showing the GST/SGT sequence as the genome protection pathway. However, the assumption the germline was segregated in EMs from somatic cells is evolutionary less correct.[69] Historically, it was the AMF germline that evolved the AMF soma and not vice versa, but it cannot be ignored that the most important function of the somatic cell line is to maintain genome integrity and restore healthy germlines and stem cell production. From this perspective, somatic cells are the unlimited reservoir for stem cell generation. In contrast to the oxygen-sensitive germline, whose genome can be altered by oxygenic stressors, the somatic genome remains intact during the SCD life period. On the other side, the formation of secondary germlines through SGTs over time leads to the upregulation of genes and the

formation of more virulent cell lines. As outlined by Yanagava et al,[70] continuous environmental stress contributes to drastic genomic changes in gene expression profiles in up to 50% of all genes and increased virulence. Such changes have been observed in periodic liver passages and liver abscesses.

As noted by Pimpinelli and Piacentini,[34] "phenotypic plasticity is the result of epigenetic mechanisms that alter gene expression in response to changing environmental conditions." Environmental changes and perturbations can cause phenotypic variation through epigenetic modulation of gene expression. They played a variety of roles in cell plasticity and adaptative evolution. This high epigenetic plasticity from somatic cells to germline can explain how wound repair occurs in metazoans from quiescent somatic cells. In the tissues of Drosophila lacking stem cells, tissue repair occurs by wound-induced polyploidization. Q cells of the Drosophila epidermis respond to injury by entering wound-induced polyploidization endoreplication. A very large polyploid cell covers the wound periphery forming small polyploid cells.[71] This is a soma-to-germ EMT-like process transiting a somatic quiescent cell to a hidden ancestral gem cell state capable both of reproductive polyploidization (RP), amitosis, and reductive daughter cell formation, followed by normal cell cycles and mitosis. Direct polyploidization of quiescent cells is unlikely. From the present information, it follows that the quiescent somatic Q cells of Drosophila use conversion mechanisms and epigenesis. The epigenetic plasticity of Drosophila somatic cells and the conversion of somatic cells into germline cells show the existence of parts of the RP/GSC cycle in Drosophila gametogenesis.[49,72,73]

The Defective SCD Germline; Loss of Function due to Homologous Recombination Deficiency

The loss of stemness and disruption of the ACD phenotype under hyperoxic conditions with an O_2 content of more than 6.0% are due to severe DNA damage, DNA double-strand breaks (DSBs), gene underfunction, and loss of function of DDR genes such as RAD50 and RAD51. Such genetic and epigenetic alterations, frequently observed in precancerous cells, are also inherited from the AMF ancestor and are known to be highly detrimental to cancer.[74]

As observed in Entamoeba, hyperoxia with more than 6.0% O_2 content converts both the germline and somatic cell line of the normal ACD phenotype to the SCD phenotype with identical daughter cells. In contrast to the somatic cell line, the hyperoxic germline shows severe permanent changes, such as loss of stemness and ACD potential caused by irreparable DNA and gene defects, but also numerous transient mitotic changes, including defective cell cycle profiles, hyperploidy of up to 40 C DNA content, spindle defects, binucleation and multinucleation, and mature and immature daughter nuclei.[39,40] When the SCD germline has more time to complete the cell cycle and divide into 2 mature daughter nuclei, it reverts to normal SCD proliferation. The subsequent cell cycle is a normal SCD cycle free of transient mitotic defects.

No irreparable defects in replication and endoreplication occur in normoxic cultures with an O_2 content of less than 6.0% or in the structures protected by a protective chitin (cysts) or actin envelope (PGCCs and aCLSs). In cysts and native PGCCs, the entire RP/GSC cycle (endoreplication, polyploidization, and reductive nuclear division) occurs without oxygen stress. Homologous recombination repair (HRR) is fully functional and DNA errors are eliminated.

Whole-genome duplication (WGD) occurring during native RP/GSC cycles (Figure 1) assumes that reproductive polyploidy generating stem cells is associated with genetic rearrangements mediated by homologous recombination (HR).[75-78] It is noted that HR is a DNA DSB repair pathway (HRR) that uses a homologous template to completely repair damaged DNA and maintain genomic stability.[79,80] As reported by Lopez-Casamichana et al,[81] "the major HRR factors of *Entamoeba histolytica* belong to the RAD52 gene group, which includes Ehrad51 and Ehrad54 and the Ehblm genes. These genes were differentially expressed when DNA DSBs are induced by UV-C irradiation or during growth stress and encystment." However, in conditions of hypoxia gene defects leading to loss of stemness and ACD phenotype disruption cannot be repaired by HR. All these defects are caused by excess oxygen and genetic and epigenetic HR deficiency (HRD).

Stewart et al[82] defined the HRD phenotype as a "phenotype characterized by the inability to effectively repair DNA double-strand breaks by homologous recombination repair (HRR), which has tremendous implications for cancer. Accordingly, genomic instability is one of the most common underlying aspects of tumorigenesis, and defective DNA repair is described as a hallmark of cancer. Failure to repair DSBs or their misrepair can result in carcinogenesis." The authors believe that biomarkers such as HR status are an important guide to treatment decisions.[82] They refer to previous work of Miller et al[83] and Heeke et al[84] who "associate the HRD pathway with multiple tumor types, including breast, ovarian, prostate, and pancreatic cancers, whereas non-HRD tumor types are referred to as HR proficient tumors." According to Marquard et al[85] and Hoppe et al,[86] "HRD can make tumors more sensitive to therapies."

Genome Repair and Germline Renewal

The mechanisms of stemness restoration and GSC generation are a legacy of the common AMF ancestor and are commonalities in the life cycle of cancer and amoebae. PGCCs and MRGSs repair DNA defects and perform DNA debris excision and genome remodeling (genome reconstruction).

Germline cell repair by MGRSs and PGCCs

To restore genomic integrity and stemness potential, the damaged germline requires nonmutational genome reconstruction. The damaged SCD germline cell fraction does not respond to SGT stimuli and does not participate in stem cell formation. Instead, the defective germline cells can fuse into MGRS. MGRSs are also known as multinucleated giant cells (MNGCs), MGCs, or PGCCs and aCLS in cancer[13,14] (Figure 3). In cultures of Entamoeba, 24-hour-old MGRSs were twice the size of normal cells, 48-hour-old MGRSs were 4 times, and 96-hour-old MGRSs were 10 times larger than normal cells in cultures (~200 μm).[41]

Kaur et al[88] found that "MNGCs (MGRSs) and homologous cell fusion can occur in cancer after irradiation. Irradiation could not destroy the ability of glioblastoma germline cells to switch into fusible phenotypes." The authors distinguish between native preexisting MNGCs from parent cell populations and the nonapoptotic MNGCs formed by irradiation and consider native MNGCs as the cause of increased resistance to therapy homotypic cell fusion as a conditioning prestage of MNGC formation.[89-91] According to the researchers above, "cell fusion processes forming non-proliferative MNGCs occur in glioma cell cultures at high frequency." The researchers also found that within a heterogeneous glioblastoma population "a small subpopulation of mononucleated radiation resistant cells (RR cells) with an innate capacity to survive the lethal radiation dose. Radiation arrests RR-cells into the G2/M phase. The non-proliferative cells are highly motile and undergo homologous cell fusion to survive the lethal radiation dose and enter a process of DNA damage repair (DDR)."

There is a close evolutionary relationship between the cysts of protists, the MGRS of amoebae, and the aCLS and the native PGCCs of cancer. PGCCs form an actin surface envelope that resembles the protective cyst wall of protists. MGRSs and PGCCs are all derived from a ur-cyst whose genetic repair capacity by HRR genes has been evolved by the AMF ancestor. MGRS syncytia arise from the fusion of hyperoxia-damaged germline cells (SCD phenotype). Of the free MGRS nuclei collected during cell fusion, some are likely to be more damaged than others. In Entamoeba, the less damaged syncytia nuclei begin RP by WGD endocycles and form the same amount of haploid daughter nuclei as the normal cyst (8 or 16) with the difference that the nuclear progeny resulting from a reproductive polyploid cycle with HR deficiency (RP+/HRD− cycle) cannot cellularize and thus cannot form GSCs. Severely damaged nuclei do not undergo polyploidization (RP negative nuclei, RP−). The whole nuclear mass, consisting of the progeny of RP+/HRD nuclei and RP− nuclei, fuses to form a hyperploid

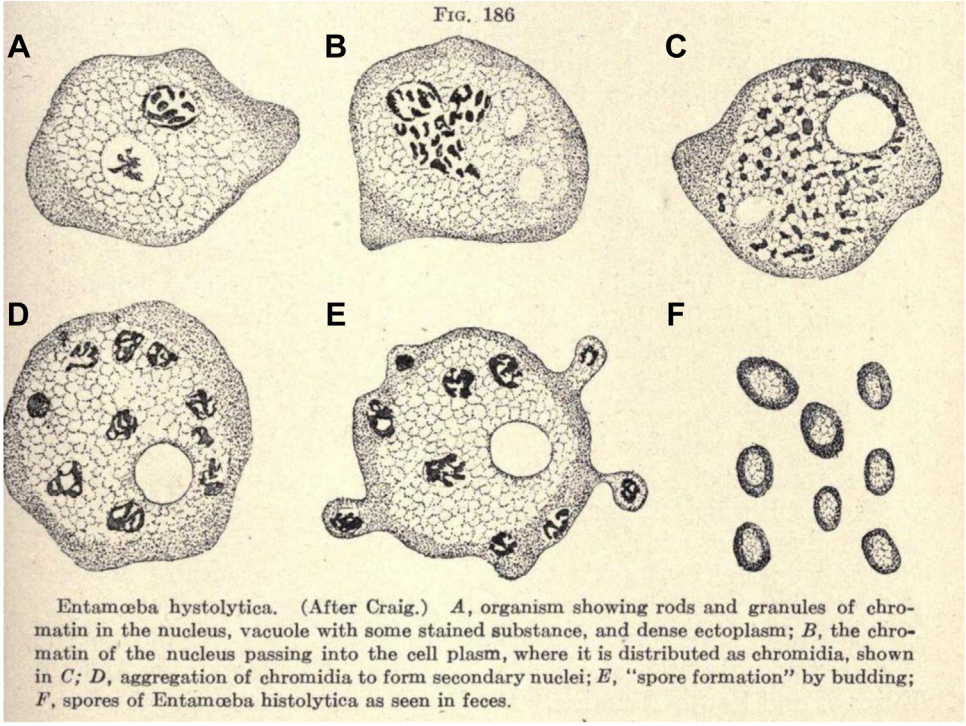

Figure 3 **Genome repair and reconstruction as discovered in Entamoeba by Craig in 1908.**[87] This ancient drawing has solved many of the PGCC puzzles of damaged glioblastoma GSCs (CSCs) on the way to restoring their genomic integrity. It shows mechanisms of DDR and genome reconstruction through giant cell formation, nuclear fusion, polyploidization, DNA debris removal, and sporulation (budding). CSC, cancer stem cell; DDR, DNA defect repair; GSC, germline stem cell; PGCC, polyploid giant cancer cell. Reprinted from https://en.wikipedia.org/wiki/Entamoeba_histolytica and Wikimedia (CC BY 2.0).

macronucleus.[92] The actual MGRS repair begins in this polyploid macronucleus, which unites the entire nuclear mass of the MGRS syncytium. When PGCC occurs in cancer without cell fusion, the nuclear mass required is generated by defective polyploidization until there is enough for genome reconstruction.

The macronucleus restores genome integrity, produces genome copies, and distributes them to the spore (bud) progeny. The spores are viable and infectious.[87] In other organisms, for example, ciliates, genome restructuring occurs by chromosome fragmentation and removal of interstitial DNA segments, DNA sequence excision, or gene scrambling (insertional mutagenesis).[93-95] The problem of damaged germline cells that lose their stemness (SCD phenotypes and HRD phenotypes) is that they cannot induce WGD and RP/GSC cycles, even when experimentally induced. As a result, the formation of cysts from defective germline cells in Entamoeba is arrested.

Genome restructuring by MGRS followed by sporulation was observed in Entamoeba by Craig[87] (Figure 3). It predicted what Kaur et al[88] found in their 2015 paper on glioblastoma. Craig's discovery of 1908 was groundbreaking and would not have been forgotten had PGCC and MGRS repair pathways not been misinterpreted as a new type of cancer cell division (neosis) from 2004 to 2006.[96,97] The graph at that is one of the mainstays of evolutionary cancer genome theory. Only based on this drawing and Craig's discovery of 1908, it was possible to understand what PGCCs are in cancer, namely a DNA damage repair (DDR) and genome reconstruction pathway. It is also proof that in cancer—similarly, with Entamoeba—-stress by oxygen excess irreversible damage the cells of the germline. Severely damaged germline cells represent an HRD phenotype without ACD potential and stemness. The damaged germline cells can recover their genome integrity only by nuclear fusion, polyploidization, and DNA debris excision, as occurs with the damaged glioblastoma germline cells (GSCs/CSCs) that fuse to form PGCCs. Because of this knowledge, it was decided to republish the old hidden drawing by Craig. Its republication serves to better understand the nature of PGCCs/MGRS processes in glioblastoma and cancer in general.

Craig saw giant amoeboid cells in stool samples and considered them to be "degenerative round cells." He suggested that unfavorable living conditions in the colon lead to such giant cells and mass sporulation. The spores occur in "enormous numbers." They had a diameter of 3 to 6 μm, a thick cell wall, were infectious, and caused dysentery in cats. Craig described the process of nuclear fragmentation and gene rearrangements in detail. He said, "the chromatin of the nucleus was distributed to the cytoplasm, while the remainder of the nucleus is absorbed or extruded. Chromidia which have been liberated in protoplasm as grains, rods, and granules … are collected in small clumps, arranging irregularly in the protoplasm or about the periphery." The final stage of repair is the cellularization: "the distributed chromatin gradually collects in small oval masses of chromatin, each surrounded by a portion of protoplasm."

Germline renewal by SGT, EMT

As reported above, the hyperoxia-induced SCD phenotype of the germline is dysfunctional and unable to continue the generation of nascent, primary CSCs. To prevent CSC depletion, signaling mechanisms of the G+S system signal the somatic, undamaged cell community to restore stemness potential. Consequently, somatic non-CSC cells induce SGT/EMT processes to form a secondary germline and new CSCs.[14] The induction of SGT/EMT processes is closely related to the previous disruption of the ACD germline phenotype and loss of stemness.

SGT/EMT processes show that EMT in cancer is an AMF cell conversion mechanism to form new healthy germlines and new CSCs. The constant switching back and forth between non-CSC (somatic) cells and secondary CSCs and germlines, and somatic cell clones reflects cancer's demand for continuous CSC production and increasing invasive potential.[98] There are differences between the primary germline, which undergoes continuous RP/GSC cycles, and the secondary germlines, which evolved from adaptative SGT processes. In Entamoeba, secondary germlines formed by encystment and HRR introduce new additional traits that can be inherited by the progeny in form of increased pathogenicity and new strain-specific markers. Such mechanisms of genome evolution by WGD and HRR originate from the AMF ancestor and were inherited through premetazoans and metazoans.

In evolution, cells exposed to environmental stress alter their expression, resulting in new phenotypes with increased pathogenicity. Altered epigenetic control of gene expression leads to different patterns of phenotypic expression with a range of morphophysiological or developmental changes. It is an evolutionary process for better-adapted offspring that fix environmentally induced traits in later generations. According to Pimpinelli and Piacentini, such variants "can become heritable via genetic assimilation processes and can also manifest as cryptic genetic variants. Heritable changes in gene expression are not associated with changes in DNA sequences but were usually triggered by epigenetic patterns."[34] Some of these patterns are completely erased and reset at the end of the mitotic cell cycle, as is the case with defective germline hyperploidy and multinucleation.

Cancer Gene Archetypes, Regulatory Networks, Hub Genes, and Multidifferentiating Germlines

From the current information, it follows that in the premetazoan period, the UC G+S was maintained as a basic cell system. On the other hand, many attempts with modified old genes or newly founded genes toward multicellularity took place. Most of these attempts were genetically unstable and led to dead ends. Consequently, the damaging gene modules were suppressed and the basic regulatory G+S gene network took control again.

Countless attempts of this sort followed, but all without much success. The product of this late premetazoan period was an enriched AMF genome with many unused genes that were later integrated into the metazoan gene networks (Figure 4). There is strong evidence that such failed premetazoan gene packages were inherited by metazoans.[7,9,14]

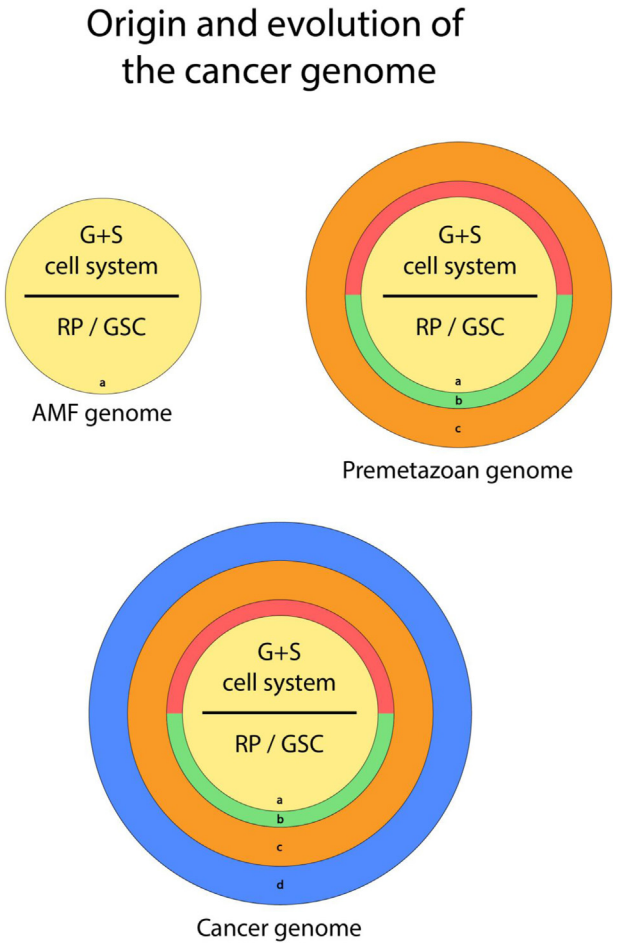

Figure 4 Cancer uses a reminiscent premetazoan genome embedded in the genome of all metazoans including humans. All amoebozoans and metazoans inherited the basic G+S genome from the common AMF ancestor (a) and thus the basic cell biological features of metazoan carcinogenesis (CSC-forming germlines and polyploidization, ACD phenotype, oxygen sensitivity, germline damage and genome reconstruction, PGCCs, EMT, and MET). The first attempts at multicellularity required a suppressor gene network against the AMF genome (b, red), but also antisuppressor genes (b, green) to return to the G+S system in case of dead ends. Genes from those failed evolutionary attempts, which were not involved in further approaches, were not discarded but stored in the genome of the premetazoans (c).[7,9] During the evolution of metazoans, which was repeatedly accompanied by cancer outbreaks, the premetazoan gene module co-evolved and was enriched by numerous antihost genes (d). ACD, asymmetric cell division; AMF, amoebozoa, metazoa, and fungi; CSC, cancer stem cell; EMT, epithelial-to-mesenchymal transition; G+S, germ and soma; GSC, germline stem cell; MET, mesenchymal-to-epithelial transition; PGCC, polyploid giant cancer cell; RP, reproductive polyploid cycle.

Hub genes, suppressor genes, and antisuppressor genes

With the advent of MC organisms, the G+S system became less and less needed. However, it was not switched off and could thus always be reactivated by stressed multicellularian cells. In this way, the G+S genome evolved in MC organisms as a counterpart to metazoan life.[14] If the G+S life cycle is reactivated by one or more weakened MC cells, a battle between old and new ensues. This was the reason why metazoans develop regulatory pathways to push back the G+S life cycle, but only to the extent that reactivation remained possible. EMs, therefore, began to balance UC and MC genes against each other, not least to keep the G+S life cycle in check for as long as possible.

In the EM era, new suppressor genes were integrated into this regulatory network, whereas old UC genes were repurposed as antisuppressor genes.[14] This regulatory "hub gene" module allowed metazoans to better control the G+S life cycle variant. As reported by Trigos et al, Erenpreisa et al, and Louka et al, in cancer there are "ancient UC and EM genes and gene subnetworks that are upregulated or downregulated during tumor development."[7,9,14,27,99] Phylostratigraphic studies have shown that in healthy cells, regulatory hub genes control the balance between UC and MC genes. Accordingly, antisuppressor genes disrupt the principles of UC/MC cooperation, especially in advanced neoplasm stages and metastases. They inactivate the MC genes while upregulating UC genes.[99]

As Trigos et al pointed out, "there are several somatic tumor genes as regulators of UC and MC subnetworks expression."[14,28] In this nodal gene network, researchers found "three UC genes from the oldest Phylostratum 1 with the cellular organization genes ACTG1, RCC2, and PKN2, five UC genes from Phylostratum 3 and 4 with the Opisthokonta and Holozoa genes PLEC, TLN1, VASP, DSP, and CTTN, and four genes from Phylostratum 5 with the early Metazoa genes ILK, CTNNA1, CTNND1, and PKP3. Because of their central role in the human gene network, these 12 genes have fundamental vulnerability and play critical roles in cancer processes associated with genomic instability, late tumorigenesis, and metastasis."[28,99] However, "it is not certain that hub genes play a crucial role in the conversion of a normal, healthy cell into malignant cells" as Martincorena et al[100] and Davoli et al[101] claimed in previous work.

Multisomatic germlines

At the end of the premetazoan period, the premetazoan genome, enriched with many additional genes, took a further evolutionary step and evolved the germline for multisomatic differentiation. This was done with the help

of the additional failed gene packages acquired during the unstable transition period. The best evidence for this is the multisomatic germline of the most primitive sponges, which has multiple somatic differentiation potentials.

According to Funayama, "the germline of sponges follows a multipotency program (GMP) capable to differentiate totipotent and multipotent stem cells. The totipotent germline cells of the sponges are the amoebocytes (archeocytes) capable of differentiation and self-renewal. They differentiate both somatic and germ stem cells (choanocytes) respectively and all types of cells constructing the sponge body respectively. Amoebocytes and choanocytes constitute the stem cell system of sponges."[48] They correspond to the premetazoan gene repertoire. Funayama considered "archeocytes as the main actor in producing all types of cells, including oocytes (but not sperm). In special circumstances, choanocytes undergo EMT to transform into archeocytes (de-differentiation and redifferentiation). Sperm and oocytes are produced from choanocytes." The data above suggest that the premetazoan gene network inherited by metazoans and exploited by the cancer has a number of additional genes that significantly enhance the invasiveness and pathogenicity of cancer.

Mosaicism and Tumorigenic Potential of CSC Subtypes

The diversification of CSCs in cancer and the ever-increasing tumorigenic potential of CSC populations occurs through the progressive evolution of somatic cell lines and clones and genomic alterations within the germline. Genetic assimilation processes could give rise to genetic variants with different patterns of phenotypic expression with a range of morphophysiological and developmental changes.[34] Such mechanisms, common to cancer and parasitic amoebae, are obviously from the AMF ancestor. In parasitic amoebae, genetic and epigenetic changes result in increased GSC potential and strains with increased pathogenicity and invasiveness.

Recently, Mitchel et al[102] considered CSCs as a cell that is plastic and modifiable by intrinsic and extrinsic factors. They understand tumor CSCs as a heterogeneous population of different phenotypes with the ability to adapt to changes in the microenvironment as well as to therapeutic stimuli. Recent work in this area suggests that CSCs resemble a mosaic. Multiple nonconvertible CSC subpopulations have been described in cancer biopsies.[103] These subpopulations have different resistance profiles. Chemotherapeutic agents may eliminate some treatment-sensitive CSC subpopulations, but the remaining treatment-resistant subpopulations rapidly fill the vacated space.[102]

Patient-derived tumor cells usually have a spectrum of transcriptional states between non–stem tumor cells and CSCs.[20] It is likely difficult to appreciate if the phenotypic and functional differences between the different phenotypes occur from individual germline clones via EMT processes or if they result from the plasticity of a limited number of clones. It was hypothesized that CSC subtypes coexisting in the same tumor originate from different niches that differentially shape the drug sensitivity of CSCs.[103,104-106] It is crucial to clarify the genetic and phenotypic characteristics of clones and GSC subtypes, therapy resistance, as well as the predicted success rates of first-line and second-line treatments.

Tumor cell heterogeneity reveals the genetic alterations occurring in tumors, molecular gene expression, and mutation profiles.[102,107,108] The researchers consider that "genetic alterations, such as EGFR and PDGFRA amplification, as well as mutations in PTEN, NF1, PDGFRA, and TP53, are common features of many tumor types." One of the most aggressive phenotypes is the mesenchymal CSC subtype frequently observed after radiation and chemotherapy and tumor recurrence. "Multiple genome alterations and mutations that CSCs accumulate during cancer progression have clinical relevance for therapy resistance and treatments."[42,102,109-111,112]

The damaged germline cells of glioblastoma are HRD cells with SCD phenotype and severe DNA defects. They lose their stemness and ACD potential and require genome reconstruction. Glioblastoma HRD cells are fusionable, form PGCC syncytia, and repair the defective RP/GSC cycles by subsequent nuclear fusion, genome reconstruction, and DNA debris excision. This pathway of genome reconstruction is homologous to the genome reconstruction described for amoebozoans.[41,87]

Conclusions

Cancer is a genome-to-genome disease. It is not an atavistic but is a complex genomic disease that does not rely on simple atavistic traits. From an evolutionary perspective, cancer is the fight of a persistent gene network originating from the premetazoan phase of life and its ancestral cell organization system against the MC host organism. It is the never-ending battle of a resurgent relic genome against misconceived dead-end cells. The progeny of this cell must bow to the dictates of the 2 strongly opposing genomes, which can cause genomic instability and multiple mutations. Moreover, the reactivated G+S life cycle can cause many defective SCD and HRD phenotypes with severe DNA defects and HR deficiency.

Experience with the life cycles of cancer and amoebae (Table 1) shows that a premetazoan gene package—consisting of G+S genes from the common AMF ancestor and additional genes from the transition era—is stored in the human genome. Evolutionary suppressor genes that evolved from the metazoan genome and antisuppressor genes that evolved from the premetazoan genes control oncogenesis. These ancient suppressor and antisuppressor

genes are the archetypes of tumor suppressor genes and oncogenes. In addition, a hub gene system consisting of premetazoan and metazoan genes provides a working balance between UC and MC gene packages. In oncogenesis, the balance (hub) tilts in favor of UC and premetazoan genes.

Phylogenomic studies support the evolutionary theory of the cancer genome. In recent years, more and more work has been done in this field, undermining the thinking of embryogenic theories and the assumption that cancer arises from early embryonic cells or embryonic stem cells.[5,8,15,48,55,113-115] The G+S life cycle of cancer is deeply homologous to the life cycle of Entamoeba. As "sister life cycles," both life cycles have helped each other to clarify their roots. The life cycle of parasitic amoebae helped to understand the life cycle of cancer, and conversely, cancer cell biology contributes to a better understanding of amoebae life cycles. Last but not the least, both life cycles show how the common AMF ancestor ensured cell system immortality—the main problem in cancer.

Immortality in cancer and amoebae is achieved by the complexity of the ancestral G+S cell system and its protective and restorative mechanisms capable of genome repair and germline restoration. Cell lines and clones have an unlimited ability to replace each other. The normoxic cancer germline has an unlimited capacity to form native CSCs through native PGCC structures (aCLSs) and polyploidization. DNA errors and polyploidization defects can be repaired by HR and HRR mechanisms. Damaged germline cells that have lost their stemness and ACD potential can be repaired by MRGS or PGCC processes. Genome reconstruction is achieved by cell and nuclear fusion and the ejection of damaged DNA material. In addition, the somatic cell line, which is resistant to oxygen, protects the germline genome under conditions of excess oxygen. All these premetazoan achievements contribute to the immortality of the G+S life cycle and cancer.

The evolutionary cancer genome theory opens new perspectives for molecular biology, cancer genetics, and cancer therapy. It points to 2 clear targets: (1) the SGT/EMT that generates new productive germline clones and the production of new nascent CSCs and (2) the native PGCCs that appear at the beginning of oncogenesis and are also involved in CSC production. The present work highlights that germline cells and CSCs are 2 distinct stages of the germline cycle and are not identical. Only healthy germlines and their ACD phenotype produce CSCs through the asymmetric cell cycle and polyploidization, whereas stem cells differentiate germ and soma cell lines and clones. In the literature, many characteristics of germline cells (ie, ACD and SCD) are often attributed to CSCs, which, however, are primarily programmed to differentiate into germ and soma cells by cell conversion and to produce new healthy germlines, clones, and CSCs.

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Conflict of Interest

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ORCID

Vladimir F. Niculescu: http://orcid.org/0000-0002-0572-2585

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