DOI: https://doi.org/10.47275/2332-4864-113
Volume 6 Issue 1
Original Article

Scholit Publishers

Journal of Clinical & Anatomic Pathology

Germline Evolution in Cancer as Explained by the Germ and Soma Theory of Dual Cell Systems

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Abstract

The present work proposes a single-cell development model for cancer based on recent insights into protist germ and stem cell biology and the analogy of the two systems. Germ stem cells (GSCs) were produced in cancer by a germline of unicellular imprinting that performs reproductive aCLS cycles consisting of self-renewing progenitor cells, committed aCLS precursor cells, aCLS polyploid cells (mother cells) and daughter germ stem cells, inherits stemness from one cell generation to the next. The highlights of this theory are:

1. The cell-of-origin of cancer activates an ancient silent single-cell genome, which can differentiate germ and soma of unicellular imprinting;

2. Germline and soma cell have differential stress resistance (DSR);

3. Hyperoxia damages the germline, which loses the ability to perform reproductive aCLS cycles and GSCs (loss-of-function), but not the proliferation capacity;

4. DNA damage signalling cells of the defective germline induce an EMT-like soma-to-germ cell transition (SGT) that increases the fitness of the CSC pool;

5. The hyperoxic damaged genome must be repaired by multinucleated genome repair structures (MGRSs) better known as "pre-existing" PGCCs;

6. Germline evolution occurs by alternating normoxic and hyperoxic damaged phenotypes; genome reorganization increases the fitness and invasiveness of CSCs;

7. Genotoxically induced PGCCs are not identical to "pre-existing" PGCCs.

Keywords: Cancer; Entamoeba histolytica; Entamoeba invadens; Reproductive cysts and cyst-like structures (aCLS); Polyploidy; Genome repair; EMT

Abbreviations

ACD: Autonomous cyclic differentiation of germ-precursor cells; aCLS: Cyst-like structure; CSCs: Cancer stem cells; DSB DNA: Double strand break; DSR: Differential stress resistance; EMT: Epithelial to mesenchymal cell transition; HGCs: Haploid germ cells; GSCs:Germ stem cells; MGRS: Multinucleated genome repair syncytia; MMT: Magna-minuta transition (Entamoeba); PGRS: Polyploid germline repair structure; PGCC: Polyploid giant cancer cell; SGT: Soma to germ transition (cancer)

Introduction

In addition to the many unanswered questions about the origin of cancer and CSCs and the evolution of cancer germlines, there is also the debate about the role of polyploidy in cancer development. I believe that we can answer many of the unresolved questions by taking a deeper look into the cell biology of protists and their dual germ and soma lines. Unfortunately, knowledge of germline and soma genomes in protists is also sparse and comes mainly from the world of ciliates and yeasts, which differentiate soma and germline nuclei [1] but include them in the same cell [2]. In contrast, the intestinal pathogenic amoebae Entamoeba histolytica and Entamoeba invadens differentiate separate germ and soma cells [3].

Over the past two decades, cancer researchers have intensively studied polyploid giant cancer cells (PGCCs) without fully unravelling their origin, function, and significance. PGCCs are specific mononucleated or multinucleated polyploid cells that appear after genotoxic treatments with chemotherapeutics or irradiation. Similar structures have also been observed in untreated cancers and called "pre-existing PGCCs". When unicellular stem cells and the dual life cycle of protists were not fully deciphered, cancer cell researchers resorted to the age-old idea of the embryonic

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Citation: Niculescu VF (2021) Germline Evolution in Cancer as Explained by the Germ and Soma Theory of Dual Cell Systems. J Clin Anat Pathol, 6(1): 113. DOI: https://doi.org/10.47275/2332-4864-113

Received: April 11, 2021; **Accepted:** April 28, 2021; **Published:** May 03, 2021

origin of cancer and tried to explain all PGCCs by the "embryonality" of tumor cells.

In 2019, a new theory about the origin of PGCCs was introduced by Liu J (2019) [4]. This "life code theory" is an embryonic theory implementing tumorigenesis in the human life cycle. It assumes that the PGCC and its numerous daughter cells are strongly related to embryonic cells. Two years ago, Liu's researcher's group introduced the term "giant cell cycle". They consider the giant cell life cycle as a continuous cycle that reprograms normal cells to give rise to high-grade tumors (de novo tumorigenesis) and subline that PGCCs have multipotent stemness. The "life code theory" underlines the similarities between "pre-existing" PGCCs and blastomeres and assumes that PGCCs express embryonic stem cell markers [4-6]. They propose that PGCCs are capable of differentiation in multiple lineages of daughter cells such as primordial germ cells and a wide spectrum of epithelial neoplasms from high-grade to benign tumors. Based on this assumption, they suggest that "the embryonic germ life- cycle" and "the giant cell life-cycle" are like twins: (i) the good twin - so far the stem cell differentiating program is blocked - generates the normal fetal development or well-differentiated benign tumors, while (ii) the evil twin doesn't progress through normal embryonic development and gives rise to undifferentiated malign tumors occurring by the de-differentiation of somatic cells.

Embryonic cancer theories [7-10] collide strongly with so-called "atavistic theories" [11-18] gaining more and more relevance in the last years. "Pre-existing" PGCCs are, in my opinion, ancient genome repair structures of unicellular imprinting that are strongly related to the polyploid genome repair structures MGRSs of Entamoeba [1], and the MNGCs (MGCs) [19]. They repair irreparable DNA DSB defects caused by excess oxygen [1]. Most "atavistic" theories view cancer as the breakdown of molecular mechanisms evolved by multicellular life. According to Nedelcu AM (2020) cancer would be the disruption of genetic networks associated with multicellularity [20]; it leads to molecular phenotypes and population dynamics similar to those of unicellular organisms [21-24]. In other words, there are a growing number of voices in favour of a unicellular view of cancer as a result of a multicellular disintegration process. More and more researchers assess cancer as "reminiscent of unicellular life" and consider it as a pre-programmed state of unicellularity conserved in multicellular organisms [25,26]. Many of the cancer cell traits are also expressed by unicellular species; at least in the early stages, cancer resembles the unicellular way of life and has many phenotypic similarities to unicellular organisms [27]. Also, it has been recognized that similar to protists, some cancer cell lines, do not respond to limiting (damaging) conditions and continue to grow instead of evolving mechanisms to avoid them (e.g. oxidative stress) [28]. This differential stress resistance (DSR) provides resistance to the toxicity of hydrogen peroxide and chemotherapeutics. In the present work, analogous data on germline development in protists are used to develop a model for cancer germline development.

Does cancer have a dual cell system?

Is there a dual cell system in cancer consisting of germ and soma? Yes, it is there and consists of cancer stem cells (CSCs) and non-CSCs. Researchers working with laboratory or collection cultures know that cancer cell cultures have two subpopulations with differential stress resistance (DSR) [29]. One is the dominant somatic subpopulation, sensitive to radiation and chemotherapeutics that dies after treatments with genotoxic agents. The second is a minor cell fraction that is resistant to genotoxic agents and survives. Almost all cancer cell lines contain this resistant cell fraction, which proliferates slowly in laboratory air cultures (hyperoxia, 21% O_2 content) or enters a state of quiescence. It disappears in some culture passages and reappears in later subcultures through a process of "epithelial to mesenchymal cell transition" (EMT). EMT activation has been associated with the development of CSCs) [30,31]. Treatments with $CoCl_2$ - a chemical that can induce sometimes a state of hypoxia - have the same effect on cancer cells as other genotoxic agents. It kills the dominant somatic cell fraction and leads the remaining $CoCl_2$-resistant cells to form polyploid giant cancer cells (PGCCs) [5].

One of the unanswered questions in cancer - which particularly irritates and has led to the present theory - is the natural "pre-existing PGCCs" in cancer, how and why they arise, and what role they have in cancer development. Little is known about this. I think the answer comes from unicellular organisms such as Entamoeba.

The dual cell system of Entamoeba consists of germ and soma

Several pathogenic protists such as Entamoeba and Giardia or free-living protists such as Colpoda have a dual cell system consisting of two distinct subpopulations. The dual life cycle of Entamoeba consists of a vegetative/ somatic subpopulation generated by the vegetative-trophic subline (soma) and a second subpopulation generated by the germline [1]. In cultures, somatic subpopulation is dominant.

For many years parasitologists have called these two different sublines "forma magna" (soma) and "forma minuta" (germ). Both phenotypes are euploid cell types with 1C DNA content.

Minuta is the germline of Entamoeba that forms in optimal living conditions reproductive polyploid cysts. The progeny of the reproductive inner cyst cell are the haploid germ cells (HGCs) that mature into stem cells. Under normoxic living conditions, the minuta germline of Entamoeba proliferates through asymmetric cell cycles and cyclic differentiation. It differentiates self-renewing minuta progenitor cells and committed cyst-precursor cells, which stop proliferation and switch to a reproductive cell cycle variant - the germ and stem cell cycle (GSC-cycle). After hatching in a second host organism, the hatched metacyst disseminates the next generation of HGCs (Figure 1).

The GSC- cycle is the developmental pathway that occurs in Entamoeba during the encystation and excystation and is interrupted by the cyst dormancy phase. All intermediate cell stages between two successive generations of reproductive cysts belong to the GSC family that pass stemness from generation to generation [1, 3]. The GSC family consists of progenitor and precursor cells, immature and mature inner cyst cells, and HGCs, which mature into stem cells. Magna is the somatic cell line; it does not form cysts and does not participate in the GSC- cycle [1].

Minuta germ cells are sensitive to excess oxygen; their natural normoxic habitat is the precapillary mucosal interface of the colon [1], where numerous aerobe and facultative anaerobic bacteria consume oxygen and establish a local oxygen gradient that meets the physiologic requirements

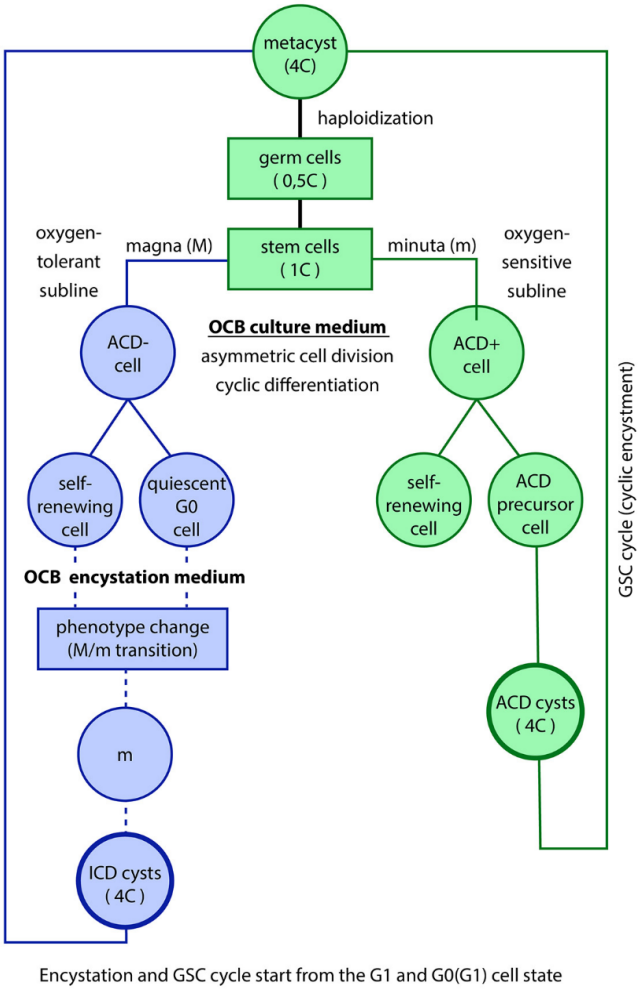

Figure 1: When stimulated to GSC cycles and encystment, damaged minuta germ cells that have DNA DSB defects cannot form cysts. Instead, they become fusible, undergo a process of homologous cell fusion, and form multinucleated syncytia termed MNGCs or MGCs [19]. Each enclosed nucleus enters a GSC- cycle and forms multiple haploid subnuclei (HSNs), but the HSNs have a defective genome and cannot cellularize into GSCs. They inherit the DNA DSB defects of the mother cell. Due to the remaining damage, the defective subnuclei fuse to form a large hyperploid nucleus. This giant hyperploid nucleus reconstitutes the germline genome and forms a high number of genomic intact subnuclei that cellularize into infectious mono-nucleated "spores" protected by a thick envelope [1]. It follows that the giant polyploid syncytium formed by homologous cell fusion is a multinucleated genome repair structure (MGRS) with enhanced reproductive cyst-like function. It repairs the germline genome damaged by excess oxygen and produces a great number of viable and genomically intact daughter cell "spores".

In this sense, the polyploid MNGCs (MGRS) of Entamoeba can be considered as reproductive cyst-like structures (rCLS). However, the progeny of normal cysts is not identically to the progeny of MGRSs. While in original cysts the GSC progeny resemble the previous GSCs, the MGRS progeny more closely resembles small mononuclear cysts (spores) and are infectious.

of Entamoeba. The transverse gut oxygen gradient varies between 0.1% and 5.7% O_2. Under conditions of deeper hypoxia, the oxygen-sensitive minuta germline survives without damage. When transferred to normoxia it returns to the fully functional normoxic cell state and re-enters the reproductive GSC- cycle. In contrast, hyperoxic living conditions and excess oxygen > 5.7% O_2 content – such as found in bacteria-free cultures, in the bloodstream, and well-oxygenated tissues - can damage the oxygen-sensitive minuta germline. Cells of damaged minuta germlines are not able to form cysts and GSCs (loss-of-function) and are unable to repair drastic genome damage and DNA DSB defects.

Reproductive germline function, intracystic 4C ploidy and replication error repair

Under normoxic conditions, minuta germ cells that proliferate via asymmetric cell cycles can enter the reproductive GSC cycle and form cysts that ensure the genomic integrity of the HGC progeny. The inner cyst cell undergoes two whole-genome duplications (WGD) rounds that access DNA damage responce (DDR) mechanisms [1]. Knowledge of the intracystic DDR mechanisms is sparse. Recently, DDR mechanisms have been described in the dormant cyst of the free-living ciliate Colpoda cucullus [32]. There is evidence that Colpoda cysts can repair DNA single-strand breaks (DNA SSB) that occur either during replication or by damaging agents [35]. Unrepaired DNA SSB can lead to the collapse of the replication forks, resulting in replication stress and double-strand breaks (DNA DSB), which are most difficult to repair [33-35]. Accurate repair requires the homologous recombination (HR) machinery and homologous DNA templates produced by polyploidization. Recent findings in macrophages show that DNA damage signalling and DDR mechanisms are directly linked to polyploidization [36-38].

Irreparable loss-of-function and vegetative hyperploidization caused by excess-oxygen

Cells exposed to prolonged excess oxygen in cultures undergo symmetric hyperploid cell cycles, have lost the GSC function, and are unable to produce cysts. The hyperoxic phenotype has difficulty with cell cycle progression and cytokinesis. It cannot repair the loss-of-function (no GSC cycles) and DNA SSB defects caused by hyperoxia [1]. To compensate for the damage, the defective minuta germline produces an excess of genome copies. The DNA content is highest in the primary hyperoxic cultures (20C-40C DNA content). It decreases by about half in better adapted longterm cultures and disappears upon return to normoxia [39, 40]. This reduces the nuclear size and nuclear volume also [39]. Hyperploidization is thought to buffer against DNA damage by providing extra copies of important repair genes [41]. In response to stress, the germline of Entamoeba develops increased genome plasticity, epigenetic changes, and compensatory protective mechanisms. They activate overexpression of DNA repair genes, DDR mechanisms, and repair networks capable of reducing DNA replication stress [42, 43].

Germline renewal by soma-germ transition (SGT)

Functional recovery (gain-of-function) comes from the oxygen-tolerant Magna subline. Hyperoxia and air cultures do not damage the somatic genome, but Magna cells cannot form cysts and do not participate in GSC cycles. However, hyperoxic somatic Magna cells may convert into minuta germ cells via soma-germ transition (SGT). The new minuta germ cells are fully functional. They can start the GSC cycle and form intact stem cells. SGT is a transition process analogous to the EMT in cancer.

Damaged germ cells can repair Entamoeba genome

When stimulated to GSC cycles and encystment, damaged minuta germ cells that have DNA DSB defects cannot form cysts. Instead, they become fusible, undergo a process of homologous cell fusion, and form multinucleated syncytia termed MNGCs or MGCs [19]. Each enclosed nucleus enters a GSC- cycle and forms multiple haploid subnuclei (HSNs), but the HSNs have a defective genome and cannot cellularize into GSCs. They inherit the DNA DSB defects of the mother cell. Due to the remaining damage, the defective subnuclei fuse to form a large hyperploid nucleus. This giant hyperploid nucleus reconstitutes the germline genome and forms a high number of genomic intact subnuclei that cellularize into infectious mono-nucleated "spores" protected by a thick envelope [1]. It follows that the giant polyploid syncytium formed by homologous cell fusion is a multinucleated genome repair structure (MGRS) with enhanced reproductive cyst-like function. It repairs the germline genome damaged by excess oxygen and produces a great number of viable and genomically intact daughter cell "spores".

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Germ and soma in cancer

The life cycle of cancer shows many similarities to the life cycle of Entamoeba, suggesting a common unicellular origin. This opens the opportunity to clarify many of the unanswered questions in cancer cell biology, based on knowledge of the germline and soma biology of protists. For example, I believe that stem cells in both cell systems are produced by the germline. Under normoxic living conditions, the cancer germline could perform polyploid cells (aCLS) analogous to the polyploid inner cyst cell of Entamoeba and reproductive aCLS cycles, analogous to the GSC cycle of Entamoeba. As previously described, the GSC cycle of Entamoeba produces multiple daughter cells with germ and stem cell function (Figure 1). The reproductive progeny of the aCLS are unicellular germ stem cells of unicellular imprinting, analogous to Entamoeba HGCs. In the present work, they are referred to as GSCs. It is unclear whether they are also haploid or not.

The Cell-of-Origin of cancer

The cell-of-origin is the cell that exits the multicellular lifestyle and activates mechanisms of the unicellular life that are conserved in the genome of multicellular eukaryotes. The cell-of-origin of cancer differentiates germ and soma sublines analogous to the inner cyst cell of Entamoeba (Figure 1). Both sublines have different DSR potential towards oxygen and genotoxic chemotherapeutics. Analogous to the Entamoeba germline, the cancer germline is oxygen-sensitive, whereas the somatic subline is oxygen-tolerant.

aCLS cycles of cancer are equivalent to the GSC cycles of Entamoeba

Under normoxic living conditions at the onset of carcinogenesis, germ and soma sublines most likely proliferate by asymmetric cell division. The asymmetrically proliferating germline differentiates self-renewing progenitor cells and committed precursor cells. To accumulate GSCs, the germline performs numerous aCLS cycles of unicellular imprinting, analogous to Entamoeba GSC cycles [27] (Figure 1). The aCLS cycle of cancer is the developmental pathway by which a committed precursor cell develops into a polyploid cyst-like structure (aCLS). aCLSs mature and produce multiple daughter GSCs with a unicellular phenotype, which in turn differentiates germ and soma. All phenotypes, described above (progenitors and precursor cells, aCLS, germ cells, and stem cells) belong to the stem cell family of cancer.

aCLSs are formed rapidly by cancer GSCs and could be observed only rarely after chemotherapeutical treatments and irradiation. They were recently detected in breast cancer MDA-MB-231 cell line after treatment with doxorubicin (DOX) (Figure 2) [44]. The researchers consider that the budding progeny of the giant amoeboid phenotypes - which can return to the mitotic cycle and restore the original cell phenotype - "usually undergo bipolar, less commonly tripolar or tetrapolar cell division immediately, sometimes even before or during the process of budding". Undoubtedly, this is the beginning of a new aCLS cycle for rapid accumulation of GSCs and reconstitution of the germline.

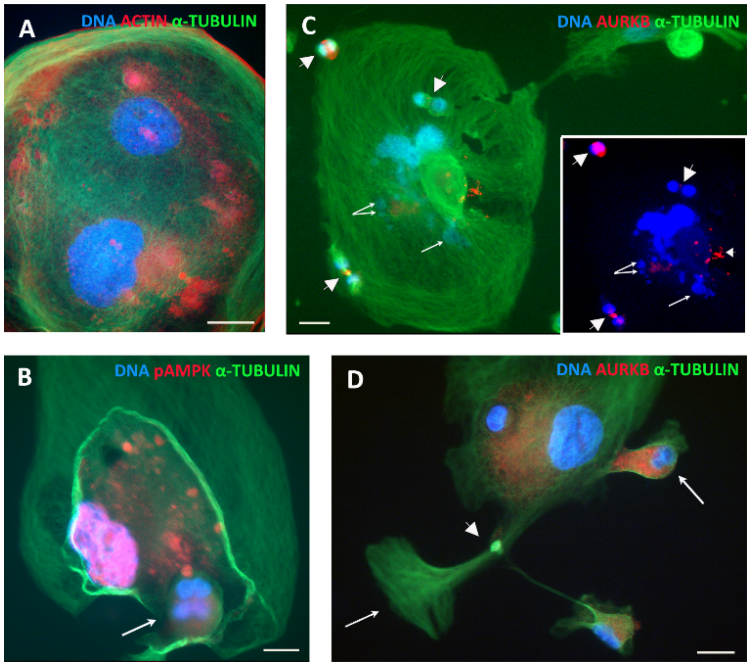

Figure 2: Genomic repair and reorganization after doxorubicin (DOX) treatment in a breast MDA-MB-231 cell line. (B) One of the mononucleated daughter cell formed by the giant amoeboid cell (arrow) enters immediately after generation an aCLS cycle and forms a typical tetranucleated polyploid cell analogous to the inner cyst cell of Entamoeba (from Salmina et al., http://www.mdpi.com/ 1422-0067/21/8/2779/s1; Reproduced with permission of the authors).

Hyperoxia abrogates cancer reproductive aCLS cycles, but not germline proliferation

Hyperoxic culture conditions damage not only Entamoeba germline but also cancer germline. In contrast to the oxygen-tolerant soma, the oxygen-sensitive germline of cancer proliferates in hyperoxic laboratory air cultures with 21% O_2 content by slow cycling. As the culture ages, the damaged germline enters a state of quiescence, while the soma cell fraction continues to proliferate and become more dominant. But even if the germline thins out considerably, it does not disappear completely. Sometimes it becomes even more abundant through an EMT-like process of soma-germline transition (SGT). SGT/EMT activation is generally associated with the development and accumulation of CSCs [27,28].

In Entamoeba, hyperoxic stress and prolonged hyperoxic conditions lead to a loss-of-function germline that is unable to perform reproductive GSC cycles. It switches into "vegetative" cell cycling, mitotic/cytokinetic dysfunction, and hyperploidization, and multinucleation [1]. Hyperoxically damaged germline cells accumulate a high genome copy number and high nuclear DNA content of 20C-40C and become multinucleated. However, the hyperploid cell state is transient, and the daughter G1 cells become mononucleated. The polyploid cell cycle can be prevented by starvation [39, 40] or return to normoxic living conditions. In adequate nutrient-deficient encystment media, the damaged germline cell transforms into a fusible and highly mobile phenotype capable of homologous cell fusion. It forms MGRSs polyploids that repair and reorganize its damaged genome.

Unfortunately, there are less comparable data on cancer. It is not known what happens when the hyperoxic damaged germline returns to normoxic culture conditions and whether it can transform into fusible phenotypes and induce MGRS. Treatments with $CoCl_2$, as performed by Zhang S, et al. (2014) are inconclusive because $CoCl_2$ (a hypoxia inducer) kills the dominant somatic cell mimicking a genotoxic agent such as chemotherapeutic agents or irradiation [45]. However, there is strong evidence that genomically damaged cancer germlines can induce a process of genome repair and reorganization by cell fusion, similar to the Entamoeba germline.

Are "pre-existing" PGCCs actually true MGRSs?

"Pre-existing" PGCCs (MNGCs) [45] have been frequently observed in untreated cancers. I hypothesize that the "pre-existing PGCCs" are distinctly different from genotoxically induced PGCCs, but are closely related to Entamoeba MGRSs that result from homologous cell fusion. Germline cell fusion was rarely observed after genotoxic treatments due to DNA DSB damage which prevents cell fusion. As a result, treated cells lose not only the proliferative ability but also the ability to change into the fusible phenotype. They become senescent and perform more or less aberrant polyploidization and repair patterns that require an unusually long time (several days) to form intact CSC-like progeny.

Kaur E, et al. (2015) found that MGRS 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 [46]. The authors distinguish between pre-existing multinucleated giant MNGCs (MGRSs) from parent cell populations and the nonapoptotic MNGCs formed by irradiation. They consider pre-existing MNGCs as a cause of increased resistance to therapy [45-48] and homotypic cell fusion as a conditioning pre-stage of MNGC formation.

According to the researchers, cell fusion processes forming non-proliferative MNGCs occur in glioma cell cultures at high frequency. It is an

innate nature of glioma cells to form MNGCs to overcome stress. The researchers found in a heterogeneous population of glioblastoma, a small population of mononucleated cells (RR cells) with an innate capacity to survive the lethal dose of radiation. 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 dose of radiation and enter a process of DNA damage response (DDR).

This process of homotypic cell fusion induced by irradiation is identical to the process of homologous cell fusion of damaged minuta germ cells observed in Entamoeba. Similarly, with the irradiated RR cells, genomic damaged minuta cells are non-proliferative and high motile; they undergo cell fusion at high frequency and form MGRSs [19].

Genotoxically induced PGCCs: polyploidization and hyperploidization without cell fusion

Chemotherapeutics including $CoCl_2$ and irradiation have much more deleterious effects on the cancer germline than hyperoxia in laboratory air cultures. In contrast to excess oxygen, chemotherapeutic agents and irradiation induce surviving germ cells into an aberrant, unnatural G2 arrest. Not all genotoxic agents have the same effect; some damage is worse than others. Many of the damaged cells undergo tetraploidization and aneuploidy while others resort to hyperploidization. Polyploidization and hyperploidization increase the number of templates required for homologous recombination. The hyperploidization observed in cultures of the breast MDA-MB-231 cell line after doxorubicin (DOX) treatment [44] is closest to the "vegetative" repair hyperploidy observed in the damaged minuta germline of Entamoeba proliferating in hyperoxic cell cultures by symmetric cell division. As mentioned above, growth in persistent hyperoxic cultures lead the germline of Entamoeba minuta into hyperploid cell cycles [1]. Hyperploidization offers a good chance through an increased number of templates capable to repair and maintain vegetative proliferation.

According to the data of Salmina K, et al. (2020), cancer cells that survive DOX treatment also hyperploidize and gradually acquire giant size, and amoeboid phenotype [44]. It is an ancestral repair mechanism that occurs both in cancer and in Entamoeba. At the end of the second week or later, the amoeboid giant cancer cells bud the progeny, which returns to the mitotic cycle. The amoeboid giant cells that restore the initial phenotype have the same function as Entamoeba MRGSs, but it achieves repair through the mechanisms of vegetative hyperploidization observed in Entamoeba.

Germline evolution in cancer: generations of GSCs of increased fitness

In my opinion, the cell of origin of cancer reactivates an archaic unicellular germ/soma life cycle that is conserved in the genome of all eukaryotes. Cancer largely retains archaic unicellular features and unicellular mechanisms that develop the CSC system. I believe that one cannot talk about the somatic evolution of cancer, without mentioning the germline evolution also.

Primary pGSCs:pGSCs arise directly from the cell-of-origin. Normoxic living conditions induce the just differentiated germline to produce daughter germ cells and stem cells of unicellular imprinting by asymmetric cell division and autonomous cyclic differentiation. The cancer germline differentiates in turn self-renewing progenitor cells and committed precursor cells capable of forming reproductive aCLS and multiple progenies (pGSCs) (Figure 3). The autonomous cyst-like structure aCLS and the aCLS cycle of cancer are equivalent to the inner cyst cell of the Entamoeba and its GSC cycle. Tetranucleated aCLSs were rarely seen in cancer cell cultures. Sometimes they could be observed after genotoxic treatments. Gentotoxically induced polyploids (PGCCs) sprout fully functional mononuclear daughter cells (spores) and these daughters start new germline and soma clones and form polyploid aCLSs and intact GSCs (Figure 2). Repeated aCLS cycles lead to the accumulation of numerous pGSCs.

In contrast, hyperoxia stops asymmetric cell division and aborts germline differentiation capacity (no GSC cycles, no aCLSs). Mechanisms of germline protection do not allow transmission of DNA damage to subsequent CSC generations. The damaged cancer germline, unable to perform reproductive aCLS cycles, is defective and awaits repair by cell fusion and MGRSs. Signalling mechanisms activated by defective germlines lacking reproductive function induce STG events and allow somatic genetic information to be epigenetically transferred into germline clones via DNA methylation, chromatin modification, or protein factors, affecting gene expression.

Secondary sGSCs: Germline damage and the lack of further pGSC production are compensated by soma-to-germ transition (STG), a process better known as EMT in cancer, and MMT in Entamoeba. Signalling mechanisms activated by defective germlines induce STG events and allow hereditary information to be transferred into germline clones [49-55]. Soma information transferred into the germline usually affects gene expression in the resulting offspring. The more effective germline clones and sGSCs produced by STG have increased fitness, leading to selection pressure in favour of sGSCs. However, hyperoxia can also damage the secondary germline and defective secondary germline clones can also induce new STG events.

Tertiary tGSCs: Hyperoxic deterioration of damaged primary or secondary germline cells can be repaired by polyploid MGRSs. MGRS can repair and reorganize the damaged germline genome and give rise to tertiary tGSCs with increased virulence and invasiveness. MGRSs, known in the cancer literature as "pre-existing PGCCs from patients", have often been observed in untreated cancer stages as a consequence of the innate germline sensitivity to excess-oxygen that occurs in excessively oxygenated tissue. MGRSs is the driver of GSC evolution [45, 47, and 48].

Germline evolution is an alternation of intact and damaged germ cell states and repair by MGRSs

The data presented in this work led to a new hypothesis about cancer's germline evolution and GSC formation. I hypothesize that the cancer germline alternates between normoxic and hyperoxic cell states (Figure 3), respectively, fully functional cell states and damaged cell states. Hyperoxic germ cells were repaired by MGRSs and the repaired cells can proliferate under normoxic conditions by asymmetric cell division and cyclic differentiation forming aCLS precursor cells, aCLS polyploids and new generations of GSCs. Germline evolution occurs in cancer through ancient unicellular mechanisms also occurring in protists.

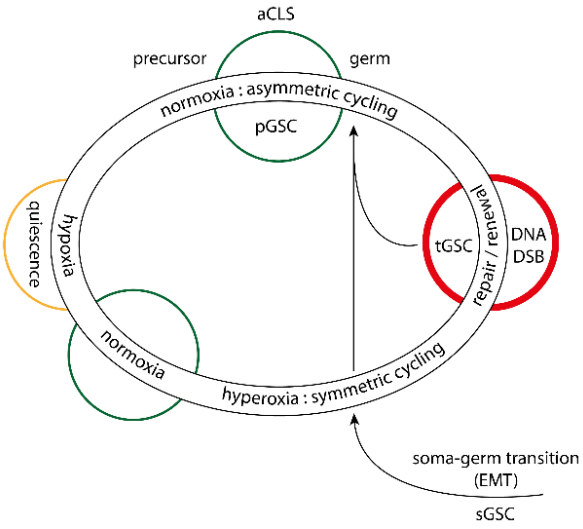

Figure 3: Cancer germline evolution. The germline proliferates under both normoxic and hyperoxic living conditions. Normoxia allows the germline to undergo numerous reproductive aCLS- cycles (green); a committed aCLS precursor cell polyploidies into an aCLSpolyploid cell that produces multiple daughter cells as primary pGSCs. The excess-oxygen in the tissue (hyperoxia) damages the germline genome. The senescent germ cells transmit information to the soma and soma cells enter an EMT-like soma-to-germ transition (SGT). Hyperoxia can also damage secondary sGSCs. Damaged pGSCs and sGSC become fusible and fuse with each other to repair and reorganize the damaged genome. MGRSs (rot) give rise to tertiary tGSCs that contain epigenetic soma information. tGSCs have increased efficiency and invasiveness.

Conclusion and Remarks

The central question that led me to elaborate on the present theory was: what do the "pre-existing PGCCs" do in stil untreated cancer, and why do they occur during cancer development? It has always been clear to me that "pre-existing PGCCs" are something quite different from the genotypically induced PGCCs occurring in treated cancers. The answer came from Entamoeba research: the "pre-existing PGCCs" are repair and genome reorganization tools capable of repairing the damaged genome of cancer's germline, analogous to the MGRSs of Entamoeba. But what are the harmful agents? Once again comes the answer from Entamoeba: it is the increased oxygen content of well-oxygenated tissues (hyperoxia), which is harmful to the germline genome. Excess oxygen leads to DNA damage and loss of function in both systems; reproductive aCLS cycles are no longer produced, and GSCs are no longer accumulated. It was clear that the polyploid inner cyst cell of Entamoeba has a natural cancer counterpart namely: the polyploid cyst-like, mother cell aCLS, which also produces GSCs daughter cells. But how does this remarkable analogy and relationship between the two cell systems come about?

Both are single-celled systems that invade the human organism. They both originate from a single cell (inner cyst cell in Entamoeba, and cell-oforigin in cancer). They have to function in both low oxygen normoxic niches and high oxygenated hypoxic tissues and are controlled by mechanisms of unicellular life. They both accumulate germ stem cells (GSCs) via reproductive-polyploid processes.

The germ and soma theory can better explain cancer evolution and the switch into unicellularity than embryonic theories and previous CSC hypotheses. This theory was developed by analogy with the germ and soma life cycles of unicellular organisms. In my opinion, the germ and soma cell system of cancer is controlled by ancient mechanisms of unicellular life that have been inherited and conserved into the genome of multicellular organisms. I think that germ and soma in cancer are differentiated by the cell-of-origin and that cancer stem cells are germ stem cells (GSCs) produced by the germline. In its initial development stage, cancer accumulates more and more primary pGSCs through repeated reproductive aCLS cycles. However, when pGSCs reach hyperoxic living conditions, hyperoxia damages the germline, which loses the capacity to perform reproductive aCLS cycles and becomes hyperoxic damaged. Hyperoxic damaged germlines are unable to restart the reproductive aCLS cycles and require genomic repair.

Loss-of-function induces soma-to-germ transition and the formation of secondary sGSC clones, which can also be damaged by tissue hyperoxia. Mixed populations of damaged pGSCs and damaged sGSCs become fusible and fuse into giant repair MGRSs that reorganize the damaged genome and include soma information. The daughter cell progeny of these MGRSs are third-generation tGSCs which is the most efficient invasive GSC generation. Germline evolution culminates in the formation of totipotent GSCs that can reproduce the entire tumor. Germline evolution from simple pGSCs to totipotent GSCs occurs through a permanent alternation of hyperoxic genome damage, SGT events, and genome reorganization.

Acknowledgements

I express my gratitude to Mr. Gregor Jaruga for the graphics and the prompt delivery.

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Addendum: The terms hypoxia, normoxia, hyperoxia are used in this paper from the point of view of the germ ine. Normoxia does not mean the atmospheric oxygen level of 21% O2, but the most physiological oxygen level below 5.7% O2 content, which allows the germline to develop all its physiological capabilities, including reproductive function and germ cell production (normoxia equals physoxia).