Cancer Genomics: Restorative Senescence, Transition to Unicellularity, DDR Circuits, and the Status Quo Ante

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Article

Cancer Genomics: Restorative Senescence, Transition to Unicellularity, DDR Circuits and the Status Quo Ante

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Abstract: All non-gametogenic germlines, including those of humans, protists and cancer, are capable of proliferation through asymmetric cell division (ACD) and stem cell differentiation, and can be classified as NG germlines or CSC-germlines. These cells are evolutionary descendants of the hypoxic Urgermline that evolved from the common ancestor of amoebozoans, metazoans, and fungi, and follow its physiological and molecular characteristics. Modern germlines and stem cells have inherited oxygen sensitivity. Stress, particularly hypoxic-hyperoxic shock, alters their genome, damages homologous recombination (HR) genes, and leads to irreparable DNA DSB, leading to the loss of functions such as stemness potential and ACD capacity. DSB-altered cells follow a pattern of unicellularization and genome repair via non-apoptotic senescence and hyperpolyploidization. Markers such as p16 and p12 characterize the dysfunctional cells. A phase of restorative senescence and unicellularization are prerequisites for cancer, but the loss and recovery of germline genome integrity also occur in unicellular organisms and parasitic amoebae. During unicellularization, human multicellular genes (MGs) are downregulated, while ancient, conserved unicellular germline (UG) genes are upregulated. Restorative senescence is an ancient cell state and genome repair mechanism, part of the cellular DNA damage response that restores dysfunctional germline cells to their "status quo ante". It reconstitutes the architecture, function, and molecular integrity of the germline genome. The cellular DNA damage repair circuitry includes tetraploidy, restorative senescence and senescence exit, proliferation through defective mitosis and symmetric cell division (DSCD), homotypic cell fusion into hyperpolyploid MGRS/PGCC structures, and genome reconstruction within giant hyperpolyploid nuclei.

Keywords: cancer; Entamoeba; genome; DDR; senescence; CSC

1. Introduction

Evolutionary cancer cell studies have demonstrated that malignancy and cancer represent more than just a remodeling process within a multicellular cell system **(1-4).** According to Evolutionary Cancer Cell Biology (ECCB), malignancy and carcinogenesis signify a transition back to a lower, unicellular state of cell organization. This unicellular system emerged over 1000 million years ago (Mya) during the transition period from unicellularity to multicellularity, involving early transitional organisms. The ancestral genome, conserved across metazoans, mammals, humans, and even parasitic amoebae, is defined by its remarkable adaptability. The ancestral genome demonstrates its ability to exploit hypoxic niches within host organisms while also retaining the capacity to repair damage caused by hyperoxic conditions. Both cancer cells and protist parasites take advantage of host environments that supply the necessary inducers to sustain their parasitic life systems, enabling survival and progression under challenging conditions.

1.1. Cell Lines and Phenotypic Heterogeneity

The unicellular cell system, which underpins the cancer cell system, evolved in the common ancestor of amoebozoans, metazoans, and fungi (AMF ancestor) during periods of hypoxia. It

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consisted of an oxygen-sensitive, non-gametogenic germline (Urgermline) and an oxygen-resistant somatic cell line **(1-3).** The Urgermline served as the blueprint for all modern germlines and stem cells, which retain oxygen sensitivity and are irreversibly damaged by hyperoxia levels above 5.7-6.0% O2 and hypoxia-hyperoxia transition. As a result, ACD lineages, including non-proliferative committed cancer stem cells (CSCs) and their CSC-producing germline (CSC-germline, ACD-germline) **(3),** exist in hypoxic conditions.

Non-gametogenic (NG) germlines proliferate through two distinct modes of cell division, each reflecting a specific functional state. Asymmetric cell division (ACD) is characteristic of the fully functional germline. The ACD phenotype represents the fully functional state of the germline, characterized by retention of stemness and its ability to produce self-renewing germline cells and committed cancer stem cells (CSCs) as sister cells. Recently, the ECCB has demonstrated that committed CSCs are not inherently proliferative; they can multiply only through cyst-like polyploidization-depolyploidization cycles or via hyperpolyploid giant cell structures, which enable the accumulation of germline progenitors through depolyploidization **(3).**

In contrast, the dysfunctional stemless germline, marked by irreparable DNA double-strand break (DNA DSB damage), undergoes symmetric cell division (SCD). This dysfunctional symmetric cell division (DSCD) results in the production of identical daughter cells that lack stemness potential and are incapable of generating CSCs. This dichotomy, with repeated alternations between functional and dysfunctional germline states, is fundamental to understanding cancer.

Germline and somatic cell lines exhibit distinct behaviors regarding apoptosis and senescence, particularly in their tendencies for apoptosis- evasion and senescence- exit. Restorative senescence and senescence exit occur exclusively in genomically damaged germline cells of protist and cancer, driven by DNA DSB signaling, and genome repair circuits, facilitating the reconstitution of a functional genome. In contrast, apoptosis is a characteristic fate of somatic cells while apoptosis-evasion is more closely associated with soma-to-germ transitions (SGT), often recognized in cancer as epithelial-to-mesenchymal transition (EMT). During a fractal EMT process - comprising somatic-like EMT-E cells and germline-like EMT-M fractions – cells demonstrate varied responses to DNA DSB damage. EMT-E cells are more prone to apoptosis failure, whereas EMT-M cells frequently achieve apoptotic evasion, enabling their return to the cell cycle. This fractal EMT process imparts a similarly fractal structure to apoptosis **(1-3).** The inability of current cancer research to elucidate the evolutionary significance of this "plasticity" phenomenon has perpetuated confusion and misinterpretations in in the study of senescence and apoptosis.

1.2. Germline Vulnerability to Hyperoxia

In both cancer and protists, hypoxia is indispensable for germline functionality, while hyperoxia serves as an ancestral stressor that causes germline dysfunction and CSC depletion. Germline physioxia is inherently hypoxic, and oxygen levels exceeding 6.0% (ancestral hyperoxia) irreparably damage germline genomes. Prolonged hyperoxia leads germline cells to lose their ACD capacity and stemness potential, transitioning instead to an aberrant phenotype characterized by DSCD.

Although individual DSCD cells cannot repair their hyperoxia-damaged genomes, they can undergo fusion to form multinucleated giant cell repair structures (MGRS/PGCC) during carcinogenesis, tumorigenesis, metastasis, or recurrence. The MGRS pathway is a typical unicellular repair mechanism that does not exist in multicellular organisms. It employs cyst-like amplification and polyploidization-depolyploidization cycles, evolutionary relics from the AMF ancestor **(1-3).**

Proliferating DSCD cells pose significant cancer risk. DNA DSBs may accumulate during successive cell cycles, yet DSCD cells can activate genome repair through the MGRS/PGCC pathway. This repair mechanism is mediated by an ancient gene regulatory network (aGRN) derived from the archaic genome compartment, which governs the transition from multicellularity to unicellularity in damaged cells, leading to cancer.

Historically, attempts to revert to unicellularity began during the early multicellular transition, when oxygen-sensitive germ and stem cell lineages encountered ambient oxygen levels exceeding 6.0 % (environmental hyperoxia). To survive and repair the dysfunctional genome, these cells retreated

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into hypoxic niches and reverted to a unicellular cell state capable of genome repair and reconstruction **(1-3).** Over evolutionary time, these oxygen-sensitive lineages of ancestral origin were no longer exposed to external hyperoxia, but instead encountered oxygen levels of bloodstream and tissues, which are hyperoxic relative to the hypoxic CSC-germline lineages of protists and cancer.

In modern multicellular organisms, non-physioxic conditions outside hypoxic niches irreversibly damage germline and stem cell DNA, necessitating genome repair and reconstruction through mechanisms such the unicellular MGRS pathway. The transition from multicellularity to unicellularity (MUT) resolves this genomic conflict but introduces a lethal conflict between transformed cells and the host organism, leading to mortality in untreated cases

The unicellular CSC-germline's capacity for regeneration is highly advantageous for cancer progression, enhancing resistance against host defenses. This study explores the topography of unicellularization and associated DNA damage response (DDR) mechanisms.

1.3. Multicellularity to Unicellularity Transition (MUT)—The Prerequisite for Cancer

ECCB and evolutionary studies show that MUT is an ancient adaptive mechanism from the early transition to multicellularity, as unstable early mechanisms with hyperoxic DNADSB damage and mitotic arrest use capacities of the ancestral genome and switch back to the stable ancestral life cycle to survive.

Unlike germlines and stem cell lineages of multicellular organisms, the unicellular cell system of protists and cancer retain ancestral genome repair capabilities, including polyploidy and hyperpolyploidy (2). These mechanisms enable the replacement of dysfunctional germlines with functional sublines and clones through cell fusion and MGRS formation, in cancer and tumors better known as PGCCs. The hyperpolyploid MGRS/PGCC genome repair pathway, restores functionality to DSCD germline phenotypes that have lost stemness and ACD potential **(3).**

In contrast, multicellular organisms lack the capacity for comprehensive genome repair, making unicellularization an essential adaptive strategy. During this process, highly evolved multicellular gene (MG) networks are largely silenced, enabling genome autorepair and fostering unicellular (carcinogenic) aggressiveness within the host **(1-3).** The MGRS/PGCC structures play a pivotal role throughout tumor progression and metastasis.

MUT conversion processes shortly termed unicellularization entails:

(i) Silencing of MGs and upregulation of ancient UGs; This undermines tissue homeostasis and promotes independent cellular behavior by deactivating networks that maintain tissue integrity;

(ii) Dysfunctional genome repair with restoration of functionality: hyperpolyploid MGRS structures act as repair hubs, restoring stemness and ACD functionality while endowing unicellularized germline cells with tumorigenic properties.

(iii) Reversion to ancestral cellular behavior**:** Cancer germline cells adopt autonomous characteristics typical of protists, escaping multicellular regulatory control.

1.4. Cycles of Genome Degradation and Repair

The cancer life cycle mirrors that of protists in its cycles of genome degradation and restoration. These cycles involve alternating ACD and DSCD lineages, stemness loss and recovery, and the formation of MGRS/PGCC structures for genome regeneration **(1–3).** ACD lineages consist of proliferative germline cells and differentiated, non-proliferating CSCs (committed CSCs) **(3).** Each ACD lineage, along with its differentiated CSCs, shares the same genome as its mother cell and self-renewing sister cell, while maintaining a distinct genomic profile compared to other ACD lineages and germline sublines. They all play a crucial role in the development and spread of cancer.

1.5. The Site of Unicellularization (MUT)

DDR processes that evolved in the AMF common ancestor are utilized by genomically damaged protists, human adult stem cells (ASCs), and cancer cells that have undergone a hyperoxic shock and sustained irreparable DNA DSB damage. When maintained in hyperoxic culture media, these

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damaged cells enter a state of prolonged mitotic arrest, also termed senescence. Cells that exit senescence demonstrate a loss of asymmetric cell division and stemnless.. Instead of ACD cycles, they proliferate via defective symmetric cell division characterized by mitotic and cytokinetic defects, polyploidy, and multinuclearity. This DSCD cell cycling is characteristic for hyperoxic-damaged unicellular organisms.

Post-senescent DSCD proliferation underscores that the restorative state of senescence, which does not exist in the cell biology of multicellular organisms, represents the site of pre-cancerous unicellularization. The MUT process is governed by the ancient gene regulatory network (aGRN), which activates UG and, in particular, DSCD genes. This state of non-apoptotic, restorative senescence resolves mitotic arrest while evading the control mechanisms of multicellular systems.

The following chapters examine the processes of apoptosis and senescence from the perspective of current cancer research, aiming to address common misconceptions through the lens of the ECCB. Commentary from the ECCB accompanies the statements of contemporary cancer research.

2. Senescence in Cancer, Protists and Metazoans

According to the ECCB, cellular senescence is a stress-induced state of proliferation arrest **(5**) that occurs in both non-cancerous and cancerous cells following severe DNADSB damage fby intrinsic stressors or harmful extrinsic agents and must decide their future cell fate based on whether they possess extraordinary repair systems or can access alternative mechanisms in a roundabout way to restore their genome to a functional *status quo ante*.

Such repair mechanisms are inherent in the unicellular genome of protists and are also tacitly preserved within the ancient genome compartment of multicellular organisms. Somatic cells have a lower likelihood of survival unless they undergo soma-to-germline transitions, whereas NG germline cells have a significantly higher chance of repairing their genome

The unusually long duration of senescence phases underscores the complexity and difficulty faced by genomically damaged cells in finding viable repair solutions. This challenge is observed not only in the cells of multicellular organisms but also in unicellular organisms, whose survival is always dependent on their environmental conditions.

While senescence is extensively studied in cancer research, the multitude of overlapping terms and definitions often fail to capture its evolutionary significance. To address this confusion, this work proposes classifying senescence into two distinct categories: apoptotic senescence and restorative, non-apoptotic senescence

2.1. Apoptotic Senescence: A Somatic Cell Fate

Apoptosis is an evolutionarily conserved cell death pathway essential for programmed cell removal and maintenance of organismal homeostasis. It responds to developmental cues or cellular stress and is regulated by the BCL-2 family of proteins, which include both pro-apoptotic and pro-survival effectors and govern the balance between cellular life and death **(6)**.

Whether programmed apoptosis represents a purely metazoan novelty absent in unicellular organisms, or if incipient forms exist in protists remains controversial. Apoptosis also raises questions in cancer, such as whether certain cells can escape apoptosis and resume proliferation. These debates have gained attention in recent years.

To date, two major apoptotic pathways have been identified: the exogenous or "death receptor" pathway, triggered by external stimuli, and the endogenous (mitochondrial) pathway, activated by intrinsic cellular mechanisms **(7,8).**

Apoptosis and apoptotic senescence are characteristic of somatic cancer and non-cancer cells that have been exposed to harmful environmental factors and chemotherapeutic agents and have suffered irreparable DNADSB damage. Cells undergoing a fractal EMT process at this time respond differently to stressors: more differentiated (more somatic) EMT-E products remain apoptotic, while less differentiated (more mesenchymal EMT-M, germline-like cells) are able to abort apoptosis and enter restorative stages of senescence.

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2.1.1. Parasitic Protists

In 2012, Proto et al. **(9)** reviewed evidence of regulated cell death pathways in selected parasitic protozoa, concluding that "that cell death in these organisms can be classified into only two major types: Necrosis and random death". At the time, molecular mechanisms for regulated cell death could not be conclusively identified in protists.

More recently, studies have described programmed cell death (PCD) in protists, including yeast **(10)** and several protozoa **(11-16).** For example, Koutsogiannis et al. in 2019 **(17**) explored why parasites like *Acanthamoeba* express proteins facilitate self-destruction. Using the aminoglycoside G418 to induce PCD, the researchers observed shape changes in *Acanthamoeba*, including rounding and contraction, with apoptotic body-like cell fragments appearing after six hours.

Rounding, cell shrinkage, intracellular ion fluctuations, mitochondrial dysfunction, nuclear and chromatin condensation, and finally disintegration with the release of apoptotic body-like particles have been documented in earlier studies on *Acanthamoeba* **(18–23).** However, early stages of programmed cell death (PCD), characterized by chromatin restructuring and nuclear vesiculation as observed in *Entamoeba*, have not been reported (**24**).

In culture, *Entamoeba* undergoes a proliferation phase lasting approximately 72 hours, followed by a stationary phase **(25–30).** After 96 hours, the cells increasingly enter senescence, eventually progressing to starvation and death. Subcultures derived from senescent cells older than seven days exhibit slower growth, with some cells attempting to exit senescence or succumbing to early death. Evidence suggests that controlled cell death may benefit parasite populations by enhancing their dissemination and promoting long-term survival **(15, 16**).

2.1.2. Metazoans and Humans

Apoptosis has evolved as a prominent cell death program in metazoans. Unlike protists and unicellular cancer cells, mammalian cells limit their replicative capacity through mechanisms like the "Hayflick limit," leading to senescence and cell cycle arrest when this limit is reached.

Severe or prolonged stress causes metazoan cells to die via necrosis or programmed cell death (apoptosis or autophagy). Krampe and Al-Rubei **(31**) noted that the first sign to cellular stress is cell cycle prolongation, with up-regulation of the transcription factor NF-kB and Bcl-2 family proteins and death receptors signaling, which transduces apoptotic signals. Early signs of apoptosis include cell shrinkage, chromatin condensation, mitochondrial depolarization and membrane blebbing. Molecularly, apoptosis involves increased expression of pro-apoptotic Bcl-2 family members and activation of caspases via death receptor ligation.

In summary, evidence suggests that programmed cell death also occurs in cell culture **(32).** Cells in culture exhibit features of both apoptosis and autophagy under nutrient deprivation **(33),** though nutrient supplementation can often prevent cell death.

2.1.3. Cancer

In recent years, the resistance of cancer cells to apoptosis has been widely accepted as a constitutive paradox. **(34)** On the one hand, apoptosis act as an anti-tumourigenic mechanism, beneficial in protecting against cancer development. On the other hand, PCD can confer pro-cancer advantages, promoting tumor survival, proliferation, and growth **[35].** Moreover, PCD may facilitate tumour expansion and evolution.

Apoptotic agents, such as the BCL-2 family of proteins, exhibit conflicting effects, inducing both partial death and simultaneous growth within pre-malignant or malignant cell populations. This contradictory phenomenon enables cancer cells to maintain survival and foster oncogenic progression despite apoptotic stimuli.

The concept of "fractional apoptosis" explains this paradox. It describes a scenario where apoptosis leads to the selective elimination of certain cells (EMT-E cells) while allowing others within

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the tumor population to survive, adapt, and proliferate (EMT-M). This dynamic contributes to fluctuating tumor states, ranging from aggressive proliferation to periods of remission, where effective tumor reduction occurs.

In cancer treatment, therapeutic efficacy hinges on achieving a balance where the rate of tumor cell death exceeds the rate of new cell formation. Understanding the apoptosis paradox is crucial for developing strategies that effectively target cancer cells without inadvertently supporting their survival or adaptive growth.

2.2. Restorative Senescence: A Germline Cell Fate

According to the ECCB, restorative, non-apoptotic senescence is a phase of mitotic arrest during which damaged non-cancerous and cancerous germline cells that have lost their function are given the opportunity to survive and revert to their *status quo ante*. Protist cells, such as *Entamoeba,* possess the necessary repair mechanisms - originating from the Urgermline and the common AMF ancestor - integrated into their genomes. In contrast, the NG germlines of multicellular organisms lack these inherent mechanisms.

From an evolutionary ECCB perspective, genomically damaged cells in multicellular organisms can access the unicellular DDR repair mechanism only under the condition of a MUT transition. This transition occurs within a specific restorative germline cell niche (RGN) under the influence of specific microenvironment inducers. Within the RGN, the MUT transition is accompanied by the activation of DDR circuitry, including the induction of SCD genes and senescence-exit. These processes enables DSCD proliferation and the formation of MGRS/PGCC structures.

After a prolonged state of senescence and reactivation of unicellular SCD genes, cells overcome the senescence barrier and proceed through the pathway of DSCD proliferation and MGRS/PGCC repair. This process of recovering genome integrity and stemness is homologous to the repair of damaged germline genomes observed in amoebae both in vitro and in vivo. The DDR circuitry via non-apoptotic senescence, DSCD proliferation and MGRS repair, is a unicellular ancient mechanism for repairing dysfunctional germline genomes repair and evolved in the Urgermline of the AMF ancestor, is a process that no longer exists in multicellular organisms.

During evolution, cells of multicellular organisms largely abandoned genome reconstruction in favor of apoptotic death programs. Ageing cells and stem cells in multicellular organisms are not designed to regenerate their dysfunctional genomes because ageing metazoans do not require new functional ACD lineages and stem cells. In contrast, indefinitely living unicellular systems, such as parasitic amoebae and unicellular cancer cell systems, exhibit a different behavior. When exposed to severe DNA DSB damage, both protists and cancers activate the ancestral repair pathway involving senescence exit and DDR circuitry.

2.2.1. Restorative Senescence in Protists

Protists, such as *Entamoeba*, exhibit transient senescence both in vivo and in vitro. *In vitro*, prolonged senescence occurs when intestinal amoebae are transferred directly from the hypoxic gut to bacteria- free hyperoxic cultures and the suboptimal hyperoxic are maintained. This axenic shock induces DNA DSB damage, pushing the cells into an adaptive senescence phase. Senescent cells with reactivated SCD genes can exit senescence and proliferate as tetraploid DSCD lineages. This hyperpolyploid state is essential for their long-term survival and proliferation under hyperoxic culture conditions. *In vivo, Entamoeba* cells that inadvertently infiltrated hyperoxic peri-intestinal tissues experienced severe DNA double-strand break (DSB) damage, followed by an extended period of senescence (**25, 26**).

When cultured under hyperoxic conditions for extended periods, these parasitic amoebae bypass senescence to proliferate as a tetraploid DSCD lineage characterized by mitotic and cytokinetic defects, multinucleation, and a mix of mature and immature nucle. Under appropriate conditions, DSCD cells become fusible, initiating a a phase of cell and nuclear fusion culminating in

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the formation of MGRS with giant hyperploid nuclei **(29,30).** These nuclei facilitate the reconstruction of damaged genomic architecture and restore genomic integrity **(1-3**).

2.2.2. Restorative Senescence in Cancer

Schmitt et al. in 2022 (5) differentiate between *"terminal senescence"* (apoptotic somatic senescence) and *"transitory repair senescence"* (non-apoptotic germline senescence) Terminal senescence, observed in both malignant and non-malignant cells, typically culminates in cell death. In contrast, a single state of transient senescence allows mitotically arrested germline cells to bypass senescence and transition to DSCD-driven proliferation, ultimately contributing to carcinogenesis.

Non-apoptotic senescence represents an evolutionary germline hallmark both in unicellular organisms and unicellularized cancer cells. It plays a critical role in DNA damage repair and cellular survival. Conversely, apoptotic senescence, which culminates in cell death, is characteristic of multicellular organisms. This form of senescence prevents reactivation of the SCD gene for further proliferation and initiates programmed cell death (PCD).

These antagonistic senescence states are referred to by other researchers as *pro-apoptotic senescence* (somatic senescence), which suppresses tumors, and *pro-carcinogenic senescence* (germline senescence), which promotes tumor progression through functional germline activities **(36-38).**

Debacq-Chainiaux et al. **(39)** highlighted the critical role of oxidative stress in inducing pro-carcinogenic senescence. Oxidative damage triggers a cascade of events, including prolonged senescence, "genomic instability", and transformation into aggressive cancer phenotypes. ECCB is not consistent with the notion of genomic instability for germline senescence escapers. This issue is discussed in detail in the following chapters

2.3. Therapy-Induced Senescence (TIS)

As noted by Schmitt et al. (**5**), chemotherapeutic drugs and radiation significantly increase the presence of senescence marker-positive cells (**40,41**). DNA damage is the most common driver of senescence in both non-malignant somatic cells and malignant germline cells, leading to an accumulation of senescent cells in various tissues **(42,43**). It induces senescence not only in tumors but, to a lesser extent and more transiently, in non-malignant tissues, with long-term implications for tissue recovery after the elimination of malignant cell populations.

A large number of active substances act as senescence inducers in preclinical models. These include alkylating agents such as cisplatin, cyclophosphamide, and temozolomide (**44-46**), topoisomerase inhibitors such as doxorubicin, etoposide, and camptothecin (**40,45-47**); γ-irradiation (**48**); and, to a lesser extent vinca alkaloids such as vincristine, have also been identified as senescence inducers in preclinical models. All these agents are known to upregulate the senescence marker p16-INK4a. According to Schmitt (**5**) "senescence is an integral effector mechanism induced by most anticancer treatments, especially those that result in DNA damage".

Therapy-induced senescence (TIS) can result in favorable outcomes if it halts cancer cell proliferation. Additionally, immune surveillance may contribute in eliminating senescent cells through apoptosis **(49).** For example, radiotherapy, a crucial cancer treatment, effectively induces senescence in several p53-proficient cancer cell types. The cell fate decision between senescence and apoptosis in response to radiation appears to depend, in part, on the presence of the PTEN tumor suppressors. For instance, radiation induces senescence in PTEN-deficient human glioma cells but induces apoptosis in PTEN-proficient cells.

2.4. Senescence and Stemness

Schmitt et al. (5) showed in 2022 that the TIS response in tumors is highly heterogeneous. This variability is less influenced by the tumor's type or origin and more by the characteristics and history

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of individual cells. However, the authors did not provide a detailed explanations for this phenomenon.

The ECCB offers critical insights that address this gap in highlighting several previously overlooked aspects: (i) the role of the functional CSC- germline, with its ACD- phenotype for producing CSCs; (ii) the loss of ACD potential and stemness due to DNA DSB damage; and (iii) the reconstruction of the DNA DSB-damaged germline genome through restorative DDR circuitry.

Earlier studies, such as those by Sabisz and Składanowski **(50)** and Was et al. (**51**), identified two distinct cancer cell subpopulations in the context of TIS: (i) a mini-fraction, comprising ≤1.5% of cells (CSC-germline cells), capable of re-entering the cell cycle after prolonged senescence, and (ii) the remaining ~98% of cells, which remain in a pro-apoptotic senescence state. Despite these valuable observations, the dual structure of cancer cell populations was not fully understood.

The ECCB clarifies that the 1.5% fraction represents the dysfunctional cancer germline, which has lost its stemness potential. Meanwhile, the remaining 98% consists of somatic cells that - by definition - lack stemness altogether. This ECCB distinction provides a deeper understanding of the dynamics between germline and non-germline fractions in response to TIS.

2.5. Senescence and Reprogramming

According to the ECCB and the present study, only the dysfunctional germline fraction has the ability to exit restorative senescence and initiate the process of functional genome regaining.

While researchers like Saleh et al. (**52**) hypothesized that "senescence is, in principle, a reversible condition, which becomes evident when essential senescence maintenance genes are no longer expressed," the ECCB suggests a different mechanism. It emphasizes partial "repair" through the activation of SCD and DSCD genes, reprogramming the germline into a DSCD lineage, which defective symmetric cell cycling.

Sabisz et al. **(50)** observed that this regrowth is associated with the emergence of CSCs in lung tumour cell populations. A decade later, Milanovic et al. **(53)** further substantiated this by showing that cells released from senescence re-entered the cell cycle with significantly enhanced, Wnt-dependent clonogenic growth potential compared to control cell populations that had undergone chemotherapy but never entered senescence. Previously senescent cells exhibited significantly increased tumourigenic potential. This is consistent with the ECCB's view that repeated restorative DDR cycles enhance growth potential, aggressiveness and pathogenicity.

Additionally, the studies mentioned above have demonstrated that while a single dose of a genotoxic agent can induce DNA damage, its effect diminishes once the majority of these damages have been repaired. However, it can take weeks following before cells to escape mitotic arrest (**52, 54**). Interestingly, cells that exit senescence retain several features associated with the senescent state. This post-senescent cell profile is dissociated, exhibiting a combination of partially preserved and partially reversed characteristics.

This interplay between senescence, repair and reprogramming underscores the putative paradox of senescence in tumor cell biology as a transient suppressor and potential enhancer of tumorigenesis. (**55)**

2.6. "Bright" and "Dark" Senescence in Cancer

Cellular senescence occurs not only in the early stages of tumorigenesis but also in advanced tumors in response to DNA damage caused by therapeutic interventions **(56)** but senescent phenotypes and tumor senescence emerges as a far more intricate phenomenon than previously thought.

According to Ou **(36),** the role of cellular senescence in cancer is highly dependent on cell type (germline or somatic) and context (functional or dysfunctional). On the one hand, senescence can prevent the expansion of premalignant cells by inducing permanent cell cycle arrest (bright senescence). On the other hand, it can reshape the tumor microenvironment (**57, 58)**, promoting stemness recovery and tumor progression, a condition known as "dark senescence" (**59,60)**.

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In general, cellular senescence is perceived by cancer researchers as a barrier to tumor development, capable of halting tumor growth or even inducing regression. However, if the senescence-mediated suppression mechanism is "compromised," cells can escape from senescence and acquire more aggressive, malignant phenotypes. The mechanisms that control senescence exit, the factors that induce cells to overcome growth arrest, and the resulting post-senescent phenotypes have not been fully elucidated by current cancer research (**61**). This ambiguous interpretation reflects the confusion stemming from historically inadequate understanding of evolutionary cancer cell biology. According to the ECCB, senescent exit is not due to compromised cellular mechanisms but rather results from the repair processes and the activation of unicellular DSCD genes during the phase of restorative senescence.

According to the current cancer knowledge, growth promotion driven by senescence-associated secretory phenotype (SASP) factors can be stimulated by various triggers. Growth-promoting effects have been observed in cultures containing senescent cells subjected to oxidative stress (**37**, **62**). In 2022, Huang et al **(63)** investigated the control mechanisms that determine the balance between pro-tumorigenic (dark) senescence and anti-tumorigenic (bright) senescence and proposed that different degrees of oncogene activation lead to different trajectories. They either suppress somatic tumor progression **(64)** or drive germline dependant tumorigenesis.

The ECBB emphasises that the mechanisms of senescence and apoptotic cell death are ancient, unicellular mechanisms that are used by cancer in a reciprocal sense: as a tumour-suppressing barrier or as a tumour-promoting mechanism. “Light“ and “dark” senescence ensure a balance in tumour expansion.

3. Molecular Insights

Most cancer researchers interpret DDR as a mechanism involving upregulation of anti-apoptotic signalling. (**65**) It has been suggested that intrinsic resistance to apoptosis can be overridden by genetic disruptions in key regulators such as p21WAF1/CIP1 or members of the BCL-2 family, emphasizing the role of senescence in supporting cancer cell survival **(66-68)**. Another hypothesis links the hyperproliferative nature of premalignant cells, to progressive telomere shortening **(69)**. Shortened telomeres are considered critical sites of DNA damage, triggering senescence through activation of the DDR signaling cascade.

While DNA DSB induced senescence and telomere shortening are linked to tumor progression**,** replicative (ageing) senescence represents a natural response to telomere shortening that occurs in normal ageing cells with each cell division. This type of senescence lead to cell death (**70**).

Other researchers have suggested that the choice between senescence and apoptosis is influenced by tumor suppressors like PTEN (phosphatase and tensin homologue deleted from chromosome Ten). For instance, studies indicate that irradiation of PTEN-deficient human glioma cells tends to induce senescence, whereas PTEN-proficient cells are more likely undergo apoptosis.

Chen et al. **(71)** studied prostate cancer driven by PTEN-deficient tumorigenesis and observed upregulation of p19-ARF, followed by p53 and p21, and senescence induction. Subsequent loss of Trp53 allowed these cells to resume proliferative capacity and undergo full malignant transformation. Similarly, in mammary tumorigenesis driven by HrasG12V oncogene expression, senescence was induced early in tumor progression **(64)**. High p16 expression was detected in premalignant cells but not in malignant counterparts, reinforcing the idea that non-proliferative senescence serves a protective role in tumorigenesis

In senescent cells lacking genome repair capabilities, such as in ageing tissues, apoptosis is the predominant cell fate path **(72)**. The extrinsic activities of these senescent cells, including the secretion of inflammatory factors and other components of the senescence-associated secretory phenotype (SASP)**,** amplify growth arrest effects. This contributes significantly to chronic age-related diseases, ageing, and inflammation-driven pathologies

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3.1. Inducers, Mediators, Effectors, and Markers

In the current cancer research, senescence represents an altered cell state with unique physiological and molecular characteristics. It is often described as a metastable homeostatic homeostatic condition triggered by various stressors and effectors. These effectors typically cause irreparable DNA damage leading to the upregulation of cyclin-dependent kinase inhibitors (CDKi) such as p16-INK4a and p21, which are considered key inhibitors ans senescence markers. These inhibitors initiate and maintain stable cell cycle arrest **(73).** Additionally, senescence-associated SASP factors, such as IL-6 and IL-8, have been implicated in reinforcing this process.

According to Schmitt et al. (5), the molecular mediators of proliferation arrest are the cyclin-dependent kinase (CDK) inhibitors p21 and p16 (aka

CDK inhibitors 1 and 2A). These inhibitors disrupt the formation of CDK-cyclin complexes necessary for cell cycle progression. (**74)** p16 acts specifically at the G1/S transition, while p21 inhibits multiple cyclin-CDK complexes, including CDK4/6-cyclin D**,** CDK1-cyclin B1**,** and CDK1/2-cyclin complexes

Accordingly to molecular studies, activation of the DDR signaling, which simultaneously induces senescence and latent stem-like reprogramming, can paradoxically compromise tumor suppression by promoting cancer cell survival and proliferation **(54,75).**

In contrast, the ECCB has demonstrated that both senescence and apoptosis are direct consequences of germline genome dysfunction (mitotic arrest). While genome dysfunction induces apoptosis in multicellular systems, it it leads to restorative senescence and genome reconstruction in unicellular systems including cancer and protists.

During restorative senescence, cancer germline cells and protist cells activate unicellular genes that control senescence exit and DSCD proliferation. However, proliferating senescent escapers (DSCD cells) lack stemness. Reprogramming to the *status quo ante* - the attainment of functional germline genome integrity with stemness and ACD potential - occurs only during the second polyploidization phase of MGRS/PGCC structures as their defective first-stage nuclei fuse to form giant hyperpolyploid nuclei (repair nuclei) that subsequently depolyploidize to progenitors for effective germline clones and sublines. **(3)**.

3.2. Sen-Mark+ cells

As cells exit senescence, they exhibit a robust proliferative DSCD capacity even while expressing high levels of senescence markers (Sen-Mark+ cells) **(73**). According to the researchers, this findings challenged previous understanding and highlights the complex interplay between senescence markers and proliferative behavior in cancer. It suggests that these Sen-Mark+ cells, may represent a distinct cell state with latent stem-like or regenerative potential, contributing to tumor progression and aggressiveness. The ECCB's counterarguments have already been described above.

O'Sullivan et al. **(73**) observed that successive rounds of error-prone replication in precancerous cells result in accumulated DNA damage and heightened genomic instability. According to the researchers, theses tumors can lead to proliferative post-senescent cells with defects in key senescence effector molecules. Such Sen-Mark+ cells are unique in that they express high levels of senescence markers while remaining proliferative. This paradox has been largely overlooked.

According to the ECCB, all senescent escapers are DSCD cells, which means that senescence markers are also markers of the DSCD phenotype. DSCD proliferation occurs not only in unicellular cells, but also in tumours. However, DSCD cells are not genomically unstable, but rather reflect genomic dysfunction. At the end of the DDR circuitry, hyperpolyploidisation/ depolyploidisation cycles restore functional genomic integrity and the *status quo ante.*

4. Relevant ECCB Statements

The present analysis redefines MUT as a major precancerous program that arises during the phase of prolonged non-apoptotic senescence. In contrast to previous ECCB hypotheses linking unicellularization to the phase of hyperpolyploid MGRS/PGCC repair (**1,2**), the present work

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highlights the crucial role of senescence in the initiation of MUT and presents it as a fundamental transition step towards carcinogenesis.

In cancer, restorative senescence is a critical phase that determines the restoration of functional integrity in the germline and stem cells. During this phase, the unicellular cancer germline can access unicellular mechanisms that restore the *status quo ante*. This phase facilitates the activation of DSCD genes, senescence exit, DSCD proliferation, and hyperpolyploid repair cycles.

This reinterpretation shifts the understanding of carcinogenic and tumorigenic development, identifying restorative senescence as the cellular state leading to MUT. By framing this process as a fundamental and conserved evolutionary response, ECCB offers a new lens for exploring cancer cell biology and potential therapeutic interventions. Growing evidence suggests that restorative senescence plays a more critical role in cancer development than previously thought and may be more important than apoptosis.

According to ECCB analyses, the phase of restorative senescence ends after the activation of DSCD genes, allowing damaged germlines to undergo defective symmetric proliferation. As long as favorable conditions persist, DSCD escapers undergo aberrant mitotic cycles characterized by features such as mature and immature nuclei, defective cytokinesis, and tetraploidy (DSCD markers). Under fusogenic conditions, DSCD cells can fuse into MGRS/PGCC structures capable of repairing the dysfunctional genome and establishing functional germline sublines and clones. These mechanisms are characteristic of unicellular cell systems and can even be observed in protist organisms such as *Entamoeba*.

In parasitic amoebae, severe DNA DSB damage triggers prolonged restorative senescence. Restorative protist senescence is particularly evident when hypoxic intestinal amoebae are transferred to hyperoxic cultures without oxygen-consuming bacteria (OCB). The amoebae enter a significant phase of senescence, followed by DSCD proliferation and increased hyperpolyploidy. In prolonged hyperoxic subcultures, they reduce their degree of polyploidy 10-20-fold. (**26**)

According to the ECCB, the phase of restorative senescence reconfigures the multicellular NG germline genome of humans and metazoans into a hybrid genome characterized by strong unicellular imprinting. Just as protist DSCD cells display resilience against apoptosis, the DSCD cells of cancer share this trait, effectively resisting cell death programms. This apoptotic resistance underscores the deep evolutionary parallels between cancer cells and unicellular organisms, reflecting a reactivation of ancestral unicellular survival strategies. By adopting these ancient mechanisms, cancer cells contribute to the persistence and progression of tumors, illustrating the profound evolutionary dynamics that play in cancer biology.

5. Accurate and Less Accurate Claimes

In a recent article, De Blander et al. **(76)** analyzed an impressive number of articles attempting to shed light on the duality of senescence onset and senescence evasion. However, in the absence of a suitable experimental model and sufficient evolutionary knowledge, most conclusions are ambiguous.

Some of these statements are highlighted below from the ECBB's perspective.

5.1. "Stem Cells Do Not Senesces"

The researchers analyzed the differences between cells that do not senesces and cells that undergo prolonged senescence. Their results suggest that the likelihood of avoiding senescence depends on the degree of differentiation of the cells. "Young adult stem cells do not senesce; they are more committed to differentiation" (**77-80**).

This finding is supported by the ECCB. As recently reported **(3),** the ACD germline phenotype gives rise to two daughter cells: a self-renewing germline cell and committed CSCs, the latter lacking proliferative capacity. Only the self-renewing sister cells can undergo cell cycles, whereas committed CSCs can only accumulate through /hyper-)polyploidization/ depolyploidization cycles, generating

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progenitor cells for germline clones and sublines. The fact that CSCs do not senesce is a consequence of their commitment. They are no proliferative and have not risk of DNADSB damage

5.2. Senescence Escaper "Requires the Acquisition of Polyploidy and Genomic instability"

Cancer research describes several forms of senescence: (i) replicative senescence (RS) or telomere-dependent senescence, characterized in fibroblasts and caused by telomere shortening; (ii) oncogene-induced senescence (OIS), triggered by the activation of oncogenes such as RAS; and (iii) therapy-induced senescence (TIS), which follows cancer treatments like chemotherapy and radiotherapy **(71,81, 82)**. All of them are activated via the p53/p21-WAF1 tumor suppressor pathway and share a reliance on genotoxic stress as the initiating factor. Accordingly, RS, OIS, and TIS are induced by DNA damage and are associated with endoreplication, polyploidy (tetraploidy), and extensive epigenetic reprogramming (**83-89).**

Some investigators believe that tetraploid cells exhibiting genomic instability can enter an aneuploidy pathway, suggesting that this process plays an important role in tumorigenesis independent of p53 status (**90-96**).

From the point of view of the ECCB, senescent escapers are not genomically unstable. After reprogramming by the DDR circuitry and MGRS/PGCC pathway, they regain full functionality and stability (status quo ante). This does not exclude genome expansion by fractal EMT processes.

During tumorigenesis, the cancer germline undergoes repetitive cycles of genomic dysfunction characterized by transient loss of stemness and recovery of stemness. These cycles occur continuously within tumors but do not result in persistent genomic instability. Genomic instability is a hallmark of somatic cell lines, not the germline.

In cancer, genomic instability manifests primarily as chromosomal instability (CIN), a state of permanent mitotic dysfunction, karyotypic abnormalities and aneuploidy. CIN, which contributes to intratumoral heterogeneity, is increasingly recognized as a biomarker of poor prognosis in various cancers, and its presence, along with aneuploidy, is associated with multidrug resistance. (**97-98).**

5.3.". Depolyploidization and Budding"

Senescence escapers are considered capable of driving polyploid cells to depolyploidize and bud **(99).** In the past, polyploid cells were considered fully differentiated cells because they could no longer divide. Unfortunately, the term polyploid does not distinguish between low (tetra-) and high (hyper-) ploidy. The budding of germline progenitor cells originates from hyperpolyploid MGRS/PGCC and not from tetraploid cells.

5.4. "Neosis—An Atypical Cell Division"

Researchers mention, multinucleated polyploid giant cells can restore proliferative capacity by undergoing an "atypical type of cell division known as neosis" **(94, 100-103).** Accordingly, "neosis" would produce daughter cells with reduced cell ploidy (diploidy) and prolonged mitotic life span **(101,102)** and is thus thought to be the "origin of senescence escapers" **(104-108).** Unfortunately, this statement is largely false.

First, the term neosis is a misnomer; It was introduced 2004-2006 by Sundarm et al. as a new type of asymmetric cell division, which it is not. Neosis is the formation of multiple spores (buds) from hyperpolyploid MGRS/PGCC genome repair structures **(1-3**) via reductive nuclear division and cellularization, and was descriebed even 1908 in *Entamoeba* by Craig. **(109)**.

Second, senescence escapers originate from mitotically arrested tetraploid cells that cease ACD cycling due to irreparable DNA DSB damage. These senescent cells enter DDR circuits and transition into DSCD lineage, continuing proliferation in a dysfunctional cell state until they undergo fusion to form MGRS/PGCC repair structures.

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6. Genomic Stability and the "*Status Quo Ante*"

DDR circuits restore germline fitness. Such processes occur in tumors **(80, 110,111)** but also in protists. According to the ECCB, DSCD germline lineages without stemness and CSC differentiation potential, are able to transform back to the productive ACD phenotype. This ability to return to the *status quo ante* and regain previous genomic architecture and stability differs from irreversible plasticity processes observed in other cell stages. Cancer germlines can repeatedly revert from a dysfunctional cell state to the fully functional state. De Blander et al. **(76)** also reveal that CSC-germlines can escape senescence and give rise to *genomically stable tumors*.

The dualistic model of tumor initiation proposed by De Blander et al. **(76)** distinguishes two tumor initiating pathways. The first involves the primary pCSCs, which are produced by the native germline (primary cancer germline). They escape senescence, and give rise to genomically stable tumors (primary tumors). The second pathway involves more differentiated EMT-E fractions, which drive genomic instability and contribute to the formation of rearranged, genomically unstable tumors.

This model highlights the dual evolution of cancer genomes from genomically stable primary tumors to genomically unstable tumors. Both pathways have ancestral ancestral origins, deeply rooted in evolutionary mechanisms of homotypic and heterotypic cell fusion.

Homotypic fusion precedes the formation of MGRS structures, facilitating hyperpolyploid genome repair, whereas heterotypic cell fusion occurs prior to the soma-to-germ transition (fractal EMT), and the generation of secondary sCSCs **(3).** It is hypothesized that heterotypic cell fusion destabilizes the stable genome of primary cancer germline cells through the random introduction of non-systemic MG genes. Many of the epithelial EMT-E fractions are highly unstable due to their hybrid genome that has captured functional MGs. However, the unkicellular SGT/EMT processes contribute significantly to genome expansion (genome evolution).

When EMT-E fractions and clones are genomically damaged by treatments, they enter the phase of restorative senescence as "more differentiated", genomically hybrid cells (**76**). According to the hypothesis of De Blander et al, EMT-E fractions tend to undergo restorative senescence, however, little is known about their fate during therapeutic treatments. Their escaper remain unstable and often progress to aneuploidy. In contrast, senescent EMT-M fractions can generate stable germline clones. They could enter DDR circuits and generate more evolved sCSCs.

7. Conclusions and perspectives.

In contrast to previous understanding of stem cells and stem cell niches, the ECCB demonstrated that the hypoxic stem cell niche includes two distinct cell types: the non-reproductive, committed stem cell, and its reproductive, self-renewing sister cell. The latter is part of the hypoxic NG germline and can generate additional committed stem cells through asymmetric cell division (**3**).

Conversely, non-proliferative stem cells give rise to progenitors for germline clones and sublineages via hyperpolyploid processes and reductive nuclear division. These closely related but functionally distinct cell types constitute specific ACD lineages within specific stem cell niches.

Upon leaving the stem cell niche, unprotected hypoxic germline cells are generally exposed to the hyperoxic O2 levels of surrounding tissues and the bloodstream, leading to irreparable DNA double-strand break (DNA DSB) damage in all cancerous and non cancerous NG germlines. If these cells fail to repair the damage, they become senescent, activate an apoptotic PCD pathway, and die. In contrast, the damaged cancer cell of origin enters senescence within a distinct restorative germline cell niche (RGN).

Within the RGN, specialized niche factors allow damaged germline cells to unicellularize through MUT processes and activate repair mechanisms characteristic of unicellular cell systems. The RGN drives sophisticated cellular and molecular DDR circuitry, supporting senescence exit and deficient DSCD proliferation. The dysfunctional DSCD cells can restore the germline genome functionality through cell fusion and MGRS/PGCC processes, generating progenitors for new NG germline sublines and clones. Ultimately, this facilitates the production of new generations of committed CSCs.

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Studies on parasitic amoebae have shown that both *in vivo* and *in vitro*, senescent germ cells can be reactivated by specific triggers to execute a DDR circuit that ultimately restores the genome to its *status quo ante*, reinstating stemness properties and ACD potential. In amoeba cultures, this inductive effect is driven by nutrient changes, despite the persistence of hyperoxic conditions.

According to the ECCB and the deep homology of all NG germlines and stem cells to the Urgermline, it is plausible to assume that human NG germline cells undergo similar MUT processes under comparable conditions. These processes activate unicellular SCD genes and DDR circuits, initiating genome repair mechanisms.

DDR circuitry and genome repair processes were hypothesized 20 years ago. However, at that time, little was understood about NG germlines and their role in generating non-proliferative committed CSCs. The NG germline was often confused with "proliferative CSC lines," while MUT and EMT processes were misinterpreted as "mutations." Earlier research referred to CSC re-growth, conflating CSC commitment with a reversible state of quiescence (**112**).

Cancer insists on an autonomous unicellular cell system of ancestral origin shared with protists such as parasitic amoebae. Post MUT, the unicellularized cancer system has three evolutionary pathways that basically promote germline genomic stability and expansion for stem cell diversity, and intratumoral heterogeneity:

(i) The cellular and molecular *DDR circuits* that includes the state of restorative senescence and returns dysfunctional and stemness-negative DSCD germlines, damaged by hyperoxic shock, to the *"status quo ante"* (genomic integrity) capable to continue CSC production

(ii) The *fractal EMT process* resulting from CSC depletion and heterotypic fusion of germline cells with non-systemic somatic cells. It leads to genomic instability and aneuploidy in the less de-differentiated EMT-E fractions; In contrast, germline-like EMT-M fractions, at the end of the soma-to-germ transition process, form genomic expanded germline clones and sublines with stemness and ACD potential, capable to generate new CSC fractions of with increased invasiveness and resistance to therapeutics (genome evolution);

(iii) *Protective mechanisms* to shield migrating germline cells from damaging hyperoxia in host bloodstream and tissues. Germline cells and CSC aggregate with oxygen-resistant cells to form clumps of cells called circulating cancer cells (CCSs), which transport the oxygen-sensitive cells undamaged into appropriate niches to develop metastatic processes. All of these mechanisms are cornerstones in the evolution of the cancer genome.

The DDR circuit is a repair mechanism from the ancestral life period, dating back more than 1700 Mya. To reactivate this repair mechanism, the multicellular genome shrinks back to a unicellular genome. Most MGs are silenced and the silenced UGs are reactivated. The ECCB calls this paradoxical genome transition unicellularization. From this point on, the transiting cell and its progeny lead an autonomous unicellular life within and against its multicellular host organism.

These new insights into evolutionary cancer cell biology (ECCB) are expected to advance our understanding of cancer and how to address it from a modern genomic perspective. Moving beyond the dominant mutation theory and focusing more on the genome – and its mechanisms for maintaining genomic stability and genomic integrity, but also genomic expansion - opens new tharapeutic possibilities. These include improved warning systems, strategies to inhibit MUT and other processes of carcinogenic transformation, and, ultimately, the development of potential cancer vaccines.

Abbreviations

ACD, asymmetric cell division, AMF, amoebozoa-metazoa-fungi; CSCs, cancer stem cells; DDR, DNA damage response/repair; DSCD, defective symmetric cell cycling; DNA DSB, double strand breaks; ECCB, evolutionary cancer cell biology; EMT, epithelial-mesenchymal transition; MG, multicellular genes; aGRN, ancient gene regulatory network; MGRS, multinucleated genome repair structure; MUT, multicellular-to unicellular transition; NG, non-gametogenic germline; PCD, programmed cell death; PGCC, Polyploid giant cancer cell; RGN, restorative germline cell niche; SGT, soma-to-germ transition; UG, unicellular genes.

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