Research Article

Introduction to Evolutionary Cancer Cell Biology (ECCB) and Ancestral Cancer Genomics

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Cancer is a complex and devastating disease that has engaged scientists and researchers for decades. Despite tremendous efforts, previous hypotheses about cancer development have not achieved significant breakthroughs. Evolutionary Cancer Cell Biology (ECCB) is a novel and emerging branch of oncological science that provides an evolutionary perspective on the origin of cancer. It reveals that the cancer genome evolved hundreds of millions of years ago, long before the multicellular organisms such as metazoans and humans emerged (1,2,3). ECCB aims to unify all evolutionary insights, hypotheses, and theories into a cohesive framework. It investigates the intricate relationship between cancer genomics and ancient pre-metazoan genes that emerge when normal cells transform into cancer cells. It challenges conventional wisdom of cancer research by suggesting that cancer could arise through intrinsic cellular mechanisms without genetic alterations and mutations (4). Furthermore, it postulates that somatic mutations are only secondary, downstream events in the process of oncogenesis.

1. Reasons and objectives of the present work

The focus of this work is to engage with the article titled "Somatic Evolution of Cancer: A New Synthesis" [1], which was recently published in QEIOS and reviewed by the author of this paper (https://doi.org/10.32388/SCECZ5). The reviewed article, hereafter referred to as the "New Synthesis," attempts to revive an older hypothesis with a new interpretation. Unfortunately, it does not take into account ECCB knowledge, and some of the comments lack expertise. Therefore, there arises a necessity to provide better information about ECCB within the context of a short clarifying article

The central premise of the "New Synthesis" hypothesis is the assumption that cancer can be traced back to intrinsic cell mechanisms without genetic changes and mutations. According to this view, somatic mutations are considered secondary in cancer. While this is an admirable idea, it has been postulated by many other researchers over the past few years, who were not acknowledged in the "New Synthesis." The proposal that the cell of origin of cancer is a normal somatic cell that undergoes a wound healing process and escapes proliferation control to engage in uncontrolled cell division is also controversial.

In contrast, ECCB demonstrates that the cell-of-origin for cancer is a defective, functionally impaired cell of adult stem cell origin. This cell type is characterized by severe DNA damage, loss of stemness and differentiation potential, and the capacity for asymmetric cell divisions [2][3][4][5]. From an evolutionary perspective, precancerous cells are more likely to be perceived as defective phenotypes that need repair. However, unconventional repair mechanisms employed reprogram the genome toward cancer.

2. ECCB Beginnings

In 2011, Davies and Lineweaver [6] proposed a surprising evolutionary explanation supported by paleontological and cancer genetics. Their hypothesis suggests that the mechanisms driving cancer have deep-rooted evolutionary origins, with some oncogenes responsible for cancer initiation dating back hundreds of millions of years [6][7][8]. For instance, the human oncogene Myc can be traced back at least 600 million years [9][10]. As the ECCB field continues to expand, it promises to illuminate the intricate interplay between ancient cellular mechanisms and processes involved in cancer development, offering fresh insights into the disease's origins and innovative treatment approaches.

Mark Vincent [11][12] took a step further and proposed that cancer should be considered an "asexual species," aligning with contemporary perspectives on asexual speciation and modern species definitions, despite the instability of the cancer genome. However, a few years later, it was found that this was not a contradiction at all [2][3][13][14].

According to Vincent, "the characteristics of the malignant phenotype are inherently primitive, and the malignant phenotype only makes sense in the context of the completely altered oceanic and atmospheric chemistry of the Proterozoic, when eukaryotic cells first appeared." Consequently, "the malignant phenotype represents a change (a de-repression) in the genetic program through an

evolutionary causal mechanism" [15][16][17]. In Vincent's view, cancer is a programmed, evolutionarily conserved life form and not a random aberration caused by mutations.

3. Evolutionary Cancer Genomics

ECCB has a are clear message: the evolution of the cancer genome predates the emergence of invertebrates, vertebrates, mammals, and humans. The roots of cancer extend back to a time before multicellularity and metazoans.

Over the past twenty years, ECCB research has expanded significantly with particular focus on the evolutionary origin of cancer genes, cancer-associated hyperpolyploidy and polyploid giant cells (PGCCs, which are not normally found in healthy humans and metazoans.

A decisive moment in ECCB research was the introduction of phylostratigraphic studies by Domazet-Lošo and Tautz [17][18][19] to determine the age of cancer genes. These researchers believed that phylostratigraphic methods could be employed to establish correlations between the origins of cancer founder genes (functional founder protein domains), and specific macro-evolutionary transitions. They posited that “the origin of complex phenotypic innovations will be accompanied by the emergence of such founder genes, the descendants of which can still be traced in extant organisms“. Their findings revealed that a significant number of protein domains associated with cancer predate multicellularity and have origins in unicellular organisms (*UC genes*). In addition, they identified a second wave of cancer protein domain emergence related to the evolutionary moments when multicellular animals emerged (*MC genes*).

Around the same time, Davies and Lineweaver [6] suggested that cancer "occurs when genetic or epigenetic malfunctions unlock an ancient 'toolkit' of preexisting adaptations, re-establishing the dominance of earlier layers of genes." They believed that comparative genomics and the phylogeny of basal metazoans (*Metazoa 1.0*) and early multicellular eukaryotes (*Metazoa 2.0*) could help identify the relevant genes in cancer and establish the order in which they evolved. This order, they argued, would offer a rough guide to the reverse order in which cancer develops, as mutations disrupt the *genes responsible for cellular cooperation*. However, the use of the term "Metazoa 1.0" or "proto-metazoa" in the sense employed by both authors remains a subject of debate.

The author of the present paper prefers the term *"pre-metazoan"* for all unicellular selfish organisms, including the common ancestor of amoebozoans, metazoans, and fungi (AMF ancestor), and *"ancient*

metazoans" for the first evolved multicellular organisms. Furthermore, the proposal by Davies and Lineweaver [6] that cancer represents an *occasional atavism* related to ancient cellular functions regulated by genes that have been largely repressed for over 600 million years does not align with the ECCB's perspective on cancer. Atavisms occasionally result from the malfunction of more recently evolved genes that suppress ancestral developments, while cancer is a widespread disease that statistically affects approximately half of the world's population [20]. A disease that potentially and statistically impacts one in two people during their lifetime cannot be classified as an atavism.

4. Cancer as a Derepression of Suppressed Archaic Genomes

According to Trigos et al. [21], cancer disrupts the normal functioning of the multicellular cell system. Researchers provide molecular evidence indicating a widespread shift towards the preferential expression of genes conserved in primitive unicellular species (UC genes). They argue that this disruption is a recurring feature in carcinogenesis and tumorigenesis. Tumors originating from different tissues with varying genetic make-ups often exhibit common cellular phenotypes characterized by persistent proliferation, suppression of cell death, and altered metabolism. The connections between the unicellular and multicellular components of gene regulatory networks (GRN) are disrupted, ultimately leading to the emergence of more primitive and proliferative cell phenotypes.

In their own words, "the coordinated expression of strongly interacting processes related to multicellularity and unicellularity was lost in tumors. UC genes were significantly upregulated, whereas genes of metazoan origin (MC genes) were predominantly inactivated." The researchers demonstrate that the de-repression of suppressed archaic mechanisms leading to malignant transformation is actively regulated by a set of 12 highly interconnected genes, serving as general drivers of tumorigenesis.

5. Comparative Genomics: The Deep Homology of Cancer to the Common AMF Ancestor

If all the evolutionary hypotheses mentioned in the last 20 years [7][8][11][12][17][18][19][21] were correct and valid, one would need to assume, conversely, that there are still primitive organisms displaying a profound genomic-phenotypic relationship with the core characteristics of the cancer cell system. It is

highly likely that related cell phenotypes should be discovered in an environmental setting similar to that of cancer cells, governed by the same environmental signals. Indeed, such a corresponding homologous cell system does exist: it is the parasitic Entamoeba cell system. These organisms are exposed to the same oxygen gradients as cancer cells and stem cells (CSCs) in humans, mammals, and vertebrates. Entamoebae primarily inhabit the intestines under conditions of hypoxia, with oxygen levels below 5.7% O2 (known as protist *normoxia*). They migrate into tissues with oxygen levels above 6.0% O2 (referred to as protist *hyperoxia*) and have a life cycle driven by extrinsic stimuli and stress, much like the regulatory stimuli and stress factors controlling the life cycle of cancer.

Entamoebae exhibit homologies to the life cycle of cancer [2][3][13][14]. Both cancer and protists cell systems have a non-gametogenic germline (NG germline) of ancestral AMF origin characterized by specific markers such as stemness, asymmetric cell division (ACD) with differentiation potential, and polyploid cell cycles. The genomics and phenotypic relationship of both cell systems – cancer and amoebae – demonstrate their deep homology to the primitive cell system of their common AMF ancestor and to each other.

6. The Deep Homologous Polyploidy of Cancer

Non-meiotic AMF polyploidy, inherited from cancer and protist cells, is confined to the non-gametogenic NG germline and does not occur in somatic cells. There are three known forms of ancestral cancer and protist polyploidy: (i) reproductive unicellular polyploidy, (ii) repair hyperpolyploidy by homotypic cell fusion of DNA-damaged cells, and (iii) the less relevant, aberrant stress polyploidy of defective symmetric cell cycling. None of these forms of polyploidy occur in healthy humans.

6.1. Cancer Single-Celled Polyploidy as a Stress-induced Asexual Reproduction Variant

This most archaic form of cyclic polyploidy is uncommon in humans and animals [13][22][23][24][25]. It originates from the reproductive cysts of the AMF ancestor and can only be expressed by the non-gametogenic ACD phenotype of cancer and protists capable of asymmetric cell division. It generates multiple daughter cells as germline stem cells and primary CSCs. Protist germ cells usually endopolyploidize to an 8-16 DNA content.

This form of polyploidization occurs in cancer from damaged NG germ cells after irradiation or chemotherapy. It develops different cell phenotypes, from tetraploid 4n to hyperpolyploid giant cells

with 128-256 DNA content. Irradiation and chemotherapy kill somatic cells but also many NG germ cells and stem cells. Those that survive are under a reproductive pressure and attempt to reactivate the undamaged parts of the reproductive polyploidization pathway. The number of duplicated DNA copies and the duration of the reproductive process depend on the level of damage. The result may be slow tetraploidization or marked hyperpolyploidization.

Details of an evolutionarely tetraploid pathway for tumorigenis initiation were recently described be Walen in 2022 [26]. It has been shown that the first fundamental step is the DNA damage, which, if severe enough, triggers mitotic skipping (mitotic sleeping). This, in turn, triggers a DDR (DNA damage response) mechanism to repair DNA. It is assumed that the seeded 4n cells are subsequently reduced to 2n cells and the 2n daughter cells duplicate their genome again. This 4n > 2n > 4n > 2n> sequence would thus be a relevant post-oncogenic cancer cell cycle leading to cell immortality in case of metastasis and recurrence.

This unusual division system, involving repeated tetraploid/diploid transitions, has been observed in mouse ascites cancer cells, as well as in ovarian cancers during metastasis, as a consequence of inefficient DNA damage repair (DDR). The lack of effective cell repair mechanisms in cells undergoing the 4n > 2n > 4n > 2n > cycle is even more evident in the case of MGRSs and their dividing single nuclei. The amitotically generated nuclear progeny must undergo a further unconventional process of nuclear fusion to form highly hyperpolyploid giant nuclei capable of repairing the DNA damage (see the next chapter).

However, in contrast to Walen, the author of the present work does not believe that tetraploid/diploid cycles can be responsible for the oncogenic transformation of normal human cells.

6.2. Genome Repair by Homotypic Cell Fusion Polyploidy

Homotypic Cell Fusion Polyploidy was developed many hundred milions years ago by the germline of the AMF ancestor Urgermline). This ancient form of repair polyploidy evolved by the Urgermline to repair severe DNA-damaged comes along *carcinogenesis* and *tumorigenesis* to repair germline cells and CSCs damaged through excess oxygen analogous to protist hyperoxia. The germline of the AMF ancestor and thus all germlines derived from it show an oxygen sensitivity to protist hyperoxia above 6.0 % O2. Hyperoxic stress damages cancer germline cells and stem cells. The defective cells lose their function - namely the capacity for asymmetric cell division with stem and differentiation potential -

but do not become senescent and retain the capacity for abberant polyploid cell cycles with multiple cell nuclei and symmetric cell division (SCD phenotype).

Classical multicellular repair mechanisms such as homologous recombination (HR) and NHEJ cannot repair the severe DNA- damaged phenotypes. The loss of function is irreversible and the damaged cells cannot restore it. This can only an ancient mechanism of cell and nuclear fusion with formation of multinucleated genome repair syncytia (MGRS) evolved by the common AMF ancestor. For this reason, defective symmeric cell cycling cells (DSCDs) become fusionable, and fuse to MGRSs. Subsequently, individual defective MGRS nuclei or their intrasyncytial defective progeny, fuse to an hyperpolyploid giant nucleus that excises the damaged DNA regions and reconstruct the genome and its genetic integrity.

In normal humans, there is a large precancerous DSCD cell family including VSEL cells, RR cells, and extragonadal GSCs [20]. In the pre-carcinogenic period, DSCD cells can reside in niches in a state of quiescence for several years. Changes in niche conditions and separate stimuli from the oxygen gradient can compel DSCDs to undergo cell fusion under the control of the conserved ancient gene regulatory network compartment, known as ancient aGRN. Within the giant hyperpolyploid MGRS nuclei, the DSCD genome can be reprogrammed for malignant primary CSCs. During *tumorigenesis* and *recurrence*, many of the oxygen-damaged NG germ cells and stem cells can fuse to form PGCC structures similar to MGRSs. Their hyperploid giant PGCC nuclei repair their DNA damage, reconstruct the damaged CSC and NG germ cell genome, and give rise to secondary, more invasive CSCs. [20].

7. Recent ECCB Findings Complet Previous Evolutionary Views

The fact that today's protists, human cancers and metazoan cancers share the same basic genome and cell biological features [2][3][13][14][20] suggests that the evolutionary "cancer-like" genome evolved before the transition to multicellularity, many millions of years earlier [27][28].

However, during the transition period to multicellularity, additional genes were incorporated, enabling both genomes of this era (pre-metazoan and early metazoan genomes) to work together effectively when environmental stimuli threatened the young and unstable multicellularity. *According to the ECCB, this ability to switch from multicellularity to a more primitive cell system is still present in the genomes of all metazoans, including humans.*

The new ECCB knowledge posits that metazoan evolution could not have occurred without the co-working of the ancient AMF genome. The development of early metazoans depended on the maintenance and functionality of the ancient AMF cell system. During the transitional period, numerous new genes did evolve, but many of the young metazoans were not viable and relied on the old AMF cell system for their survival. Consequntly, many of the "dead-end" genes were not discarded but incorporated into the transition genome for a possible later onset.

Furthermore, it was the ancient gene regulatory networks (aGRNs) that assumed control over the genes of both mutual cell systems. This period saw the emergence of suppression and antisuppression mechanisms, which must be associated with the so-called "de-repression of suppressed primitive transcriptional program" [15][16][17]. The suppressor-antisuppressor gene network, along with the additional dead-end genes [27][28] is homologous to the aGRN of the transition period. These genes were previously described by Domazet-Lošo and Tautz [17][18][19] as the "second gene peak." It is also conceivable that the 12 oncogenes (hub genes) genes discovered by Trigos et al. [21] primarily consisted of suppressor and de-repressor genes from the transitional period.

8. Final Consideration and Perspectives

The main messages of the ECCB are as follows:

1. Malignant transformation involves the relocation of a DNA-damaged multicellular cell of stem cell origin (DSCD cell) to a much deeper evolutionary genomic compartment controlled by the ancient aGRN network of pre-metazoan and early metazoan origin;
2. Polyploidization and hyperpolyploidization, which have their roots in pre-metazoans but are not directly relevant to humans and animals, nonetheless, play a significant role in malignancy, carcinogenesis, tumorigenesis, stem cell production, and the repair of DNA-damaged stem cells.

A comprehensive review on PGCC cells and various aspects of polyploidy and hyperpolyploids in cancer has recently been published [29]. It meticulously examines and discusses the efforts of several Polyploidy-Focused Research Groups spanning the last 15-20 years, along with the hypotheses and theories that have emerged from their work. Noteworthy contributions come from researchers such as Jekaterina Erenpreisa, Razmik Mirzayans, Vladimir Niculescu, Ken Pienta, Kristine Salmina, Kirsten Walen, Dan Zhang, and Jing Zhang. Additionally, the works of other notable scholars like Olga

Anatskaya, Aurora Nedelcu, Andreij Kasperski, David Diaz-Carballo, Mariano Bizzari, Fiorenza Ianzini, Vladimir Vinnitsky, and many more deserve acknowledgment.

It's important to note that many of these authors have put forth alternative perspectives on cancer genomics, which, due to space constraints, cannot all be encompassed in this concise introductory paper. Readers interested in exploring these viewpoints can refer to recent reviews, such as the one by Jekaterina Erenpreisa et al., titled "Advances in Genome Regulation in Cancer"[30].

It is hoped that the in-depth exploration of cancer from an evolutionary perspective can lead to novel approaches, particularly new ideas for the prevention and management of this unconventional disease.

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