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The Enigma of Cancer Resistance to Treatment

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Abstract

Polyploid giant cancer cells (PGCC) are evaluated by histopathologists for cancer diagnosis, yet their role in cancer is poorly understood. In this essay, we highlight a particular aspect of these cells in relation to genomic self-organisation and transcriptional networks with relevance to treatment resistance. Embodying dynamic restructuring of the genomic network, epigenome and microenvironment, through explorative adaptation in response to sublethal challenge these cells operate at the edge of chaos and order. This state is manifested through oscillations in opposing cell fate pathways, with accelerated senescence coupled to reprogramming and an atavistic shift towards phylogenetically ancient unicellular genetic programs accessed through bivalent mediator genes. It recapitulates certain unicellular life-cycles in a cancer "life-cycle" which reciprocally connects the somatic mitotic cell cycle with the germline cycle of the PGCC.

Keywords: polyploidy giant cancer cells, mitotic slippage, cell senescence, reprogramming, self-organization, cancer

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1. How Are Malignant Tumors Inherently and Secondarily Resistant to Anticancer Drugs?

Polyploid giant cancer cells are assessed by histopathology for cancer diagnosis, yet their biological role in cancer is poorly understood and undervalued. The special issue in Seminars in Cancer Biology is devoted to these cells and different aspects of this emerging new field of cancer biology (Liu, Erenpreisa & Sikora 2021). Our article in this issue "Paradoxes of cancer: survival at the brink" (Erenpreisa *et al.* 2020) reviews the problems associated with cancer resistance to treatments under the lens of a "cancer life-cycle" (Erenpreisa & Cragg 2007) developed by us over two decades of research. In the present essay, we highlight a particular aspect of these cells relating to self-organization mechanisms which we believe under-pin the inherent and secondary resistance to anticancer treatments.

In spite of the huge diversity in the cellular origin of malignant tumors, all aggressive cancers ultimately evolve towards a similarly invasive EMT phenotype heralded by resistance to both genotoxic and targeted therapeutics (Pienta *et al.* 2020). Massive cell death is commonly seen in the first week after such treatments, followed by subsequent disease relapse. This begs the question "How are malignant tumors inherently and secondary resistant to these treatments?"

2. Explorative Adaptation at the Brink of Catastrophic Damage

Here we present a potential mechanism termed "explorative adaptation" which paradoxically is initiated in cancer cells only "at the brink" of catastrophic damage. Differentiated somatic cells are continually adapting to small fluctuations in their environment within the context of their deterministic genetic programs and die if these fluctuations elicit changes above the established threshold. In contrast, cancer cells can adapt to unforeseen environmental challenges using exploration by "trial and error", at the edge between order and chaos (Erenpreisa & Giuliani 2019). Faced with potentially lethal damage, they begin scanning their gene networks, revisiting hidden transcriptional configurations preserved in the mammalian genome memory spanning the 3.5 billion years of cellular evolution, which has survived catastrophes resulting in extinction of up to 75% of species. Such regulation by stochastic chance and vestigial transcriptional programs (termed also "predetermined chaos") seems the most likely way to facilitate the rare escape of "lucky" survivors from near-lethal damage. Uncertainty, fluctuations, duality of opposites involved in an intensive "dialogue with the environment"—the components of "self-organization" are the main features of this regulation. These concepts stem from the thermodynamics of unstable open systems discovered by Ilya Prigogine, the 1977 Nobel Prize winner in chemistry (*The Nobel Prize in Chemistry 1977*, n.d.). Studies of this nature demand relevant experimental settings, as this kind of regulation does not conform to the expected linearity between the severity of an applied drug and the final effect on cancer. This is perhaps why the reductionist gene-centric approach of targeted therapy has largely proved a failure during the 50 years of the "war on cancer" (Weinberg 2014; Bizzarri 2017; Brock & Huang 2017).

3. Cellular Senescence Coupled to Stemness Serves as a Tool of Explorative Adaptation

The process of explorative adaptation begins with premature cell senescence (induced by oncogenes, oxidative stress or anti-cancer drugs). Senescence, which interrupts proliferation and seemed initially a desirable outcome of anti-tumor therapy, paradoxically has now been established as a gateway to genome reprogramming and cancer cell survival. However, it acts not only in a paracrine manner through the senescence-associated inflammatory secretome as initially considered. It also opens the door for multipotency (stemness) allowing explorative adaptation of the genome in the stressed cancer cell. The period of premature cell senescence lasting for days, weeks and even months after the initial insult, is also characterized by heterogeneous dual phenotypes marked by the concurrent expression of opposing regulators, pairing senescence and stemness (self-renewal), through molecular regulators such as p21 versus OCT4 and Nanog versus p16INK4a (Erenpreisa *et al.* 2020). Expression of p16, in turn, supports the reprogramming loop of the inflammatory cytokine IL-6 (Mosteiro *et al.* 2018). Physical oscillations manifest between these opposing states, literally between immortal life and terminal death. An immediate consequence of this mechanism is that traditional drug screening assays, such as 3-day viability tests, may be uninformative for treatment outcome many weeks afterwards whereas a clonal assay may be much more appropriate (Mirzayans, Andrais & Murray 2017).

4. Reversible Polyploidy of Giant Cancer Cells Provides a Platform for Clonogenic Survival

Senescent cancer cells, particularly those with defective/absent TP53, while temporarily interrupting cell divisions after genotoxic treatments, usually do not interrupt DNA replication and thus shift through aborted mitosis ("mitotic slippage") into transient polyploidisation cycles. The polyploid state has the advantage of increased gene dosage, tolerance to apoptosis, toxicity, and immunity evasion. Interestingly, IL-6 activates embryonic stemness during the initiation of PGCCs and can reprogram normal fibroblasts into cancer-associated fibroblasts (Niu *et al.* 2021). Moreover, the PGCC can undergo epigenetic diversification of their subnuclei, even redistributing DNA damage into those undergoing autophagy and then executing asymmetric division to remove damage, followed by symmetric division of the repaired daughter cells (Erenpreisa *et al.* 2017). Polyploidy through senescence provides cells with additional options and

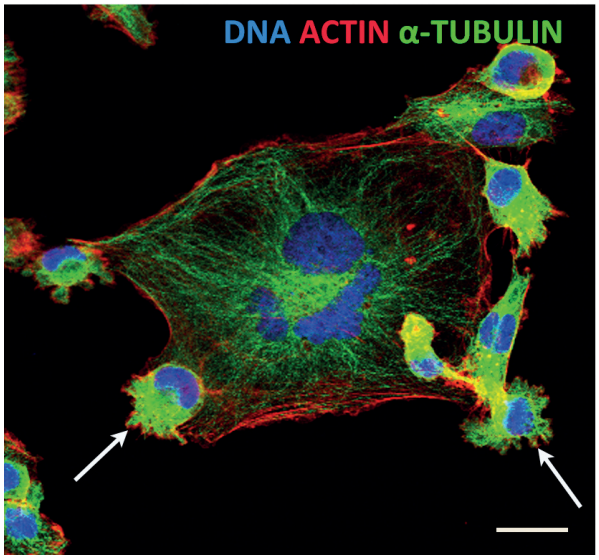

Figure 1: Budding of mitotic progeny starting mitotic divisions from a polyploid giant cancer cell (PGCC). MDA MB 231 cancer cell after 20h doxorubicin treatment, on the 7th week. Bar=25 µm (republished from Salmina *et al.* 2020).

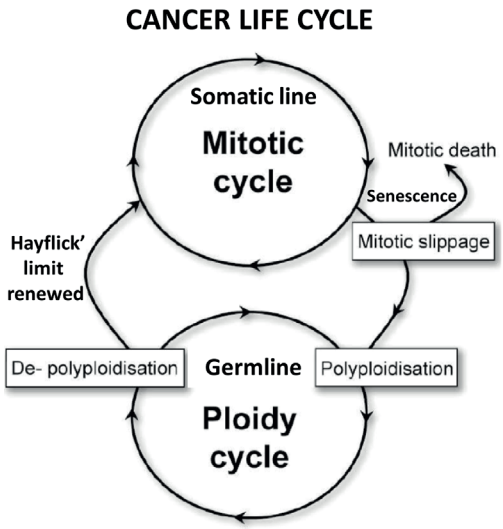

Figure 2: Schema of the "cancer life-cycle" reciprocally uniting through senescence and mitotic slippage the mitotic and the ploidy cycle of giant cancer cells (modified from Erenpreisa & Cragg, 2007).

time for repair, including recovery of the telomere attrition by alternative telomere lengthening (Salmina *et al.* 2020). Finally, this process raises the conditions for depolyploidisation and the return to the mitotic cycle by budding, which immediately starts mitotic divisions (from two weeks to several months after treatment) as seen in Figure 1.

This behavior is akin to the life-cycle of certain unicellular organisms alternating between a vegetative and generative phase with cycling polyploidy; the latter supporting the immortality of the former by renewing the Hayflick limit of telomere shortening (Figure 2). These life-cycles are likely recapitulated by cancer cells from the unicellular phylogeny.

5. Evolutionary Origin of "Cancer Life Cycle" from Unicellular Organisms

The phylogenetic origin of gene ancestry helps to understand how this can occur. The human genome possesses nearly 23,000 genes; 60% of them provide essential cell functions (DNA replication, DNA damage repair, RNA synthesis, ribogenesis, etc.). These highly conserved genes appeared in Prokaryotes and unicellular forms. Subsequently a relatively small number of new genes were added at the transition to multicellularity, where transient polyploidy and syncytia played an important role. The Cambrian explosion (ca. 500 million years ago, with atmospheric oxygen raising over 5%) was heralded by a burst of many, complex, multicellular species, while humans (the last in this 16-18 phylostrata range) contributed only an additional 0.3% of new genes.

Recent studies of gene phylostratigraphy testified that human cancers have an imbalance in the expression of these various genes—i.e. "old" genes of unicellular-origin are overexpressed, while "new" genes belonging to more recent phylostata coding for intercellular communication and complexity of higher multicellular organisms are underexpressed. The underlying rationale is that aggressive, high-grade cancers obtain some unifying phenotype present also within unicellular organisms, with mesenchymal and amoeboid features (Trigos *et al.* 2017). Moreover, the so-called cancer driver proto-genes appeared in evolution mostly in early multicellular organisms and even earlier (Domazet-Lošo & Tautz 2010). Therefore, their dysfunction in cancer may cause an imbalance between the unicellular and multicellular parts of the human genome network, collapsing it towards a more densely wired unicellular core (Trigos *et al.* 2018). Furthermore, polyploidy as such, particularly instigated by hyper-activated non-mutant *c-myc*, which also epigenetically "opens" the chromatin for multiple targets and particularly by activating bivalent genes—capable to quickly shift from poised to active state—was shown to shift cells to unicellular and cancer-linked gene ontology modules

(Anatskaya *et al.* 2020). Therefore, *myc*, this well-known stemness master gene (one of the Yamanaka reprogramming transcription factors (Takahashi & Yamanaka 2006) is an important player for inducing cancer by self-organization, coupling polyploidy with stemness through senescence (which in turn, may be also induced by over-expressed (often mutant) *ras*-family gene, the oncogenic partner of *c-myc*). It is no surprise then, that knockdown of *c-myc* in mice can cure even metastatic cancer (Morton & Sansom 2013).

6. Cancer Aneuploidy: Order from Chaos or Chaos from Order? The Role of Meiosis

Cell fate change by self-organization of the whole genome raises the question of how genomic aberrations, such as aneuploidy, fit into this model. Aneuploidy should interfere with cell division and accumulate lethal mutations, restricting tumors, but in fact accompanies aggressive cancers and is their hallmark. The most general solution of this conundrum is that polyploid genomes are unstable and lead to aneuploidy and that explorative adaptation (by trial and error) is impossible without the variability initially produced by the ensuing genome chaos. This stress-induced chaos for cell fate change often occurs in one cell division (chromothripsis) (Ye *et al.* 2018), which may result from or during mitotic slippage. By contrast, in the longer time-course, the recovered mitotic clones with aneuploidy can undergo stepwise selection of mutants more fit for survival. This represents a satisfying solution reconciling genome chaos with embryonality through "McClintock heredity" and the atavistic features of PGCCs (Liu 2021).

Another facet of this problem is an understanding of the role of the germline (primordial germ and meiotic) genes—which are also a hallmark of cancer (Bruggeman *et al.* 2018) and diagnostic or prognostic for certain cancer types (e.g. *MOS*, *SCP3*, *SOX11*, and *DMC1*). Meiotic genes (at least some of them) are also up-regulated during polyploidy in response to genotoxic stress. Although they may facilitate aberrant mitosis they may also drive non-conventional meiosis and parasexual processes coupled with polyploidy and thereby help to counteract the loss of heterozygosity (Salmina *et al.* 2019; Archetti 2020). In conclusion, these largely overlooked PGCCs, may represent a bridge between many of the paradoxical observations seen in cancer and its resistance to treatment. Hopefully, a greater focus on systems-wide understanding will help unlock deeper understanding of cancer and thereby more effective means to treat it in the forthcoming decade.

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