

Molecular and Cell Biological Considerations in the Initiation and Development of Sporadic Non-Hereditary Solid Cancers

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Abstract

This paper reviews the state of cancer research in the post-mutation era. It presents cancer as a highly complex disease viewed differently by scientists from various research fields. Histopathologists considered cancer as a disease of cell differentiation, cancer cell biologists overestimated the causal role of accumulated DNA mutations. More recently molecular biologists have focused on driver genes and driver mutations, regulatory gene networks and deregulation of the genomic balance between unicellular and multicellular gene sets (UG/MG balance). From a developmental biological standpoint, there is a clear analogy between the reproductive life cycles of cancer and protists. The key player of both analogous life cycles is the polyploid cyst, the atavistic cyst-like structure aCLS (PGCC). In the analogy to protists, we assume that the first aCLS initiating cancer originates from a mitotically blocked cell (cell of origin of cancer, protoprecursor) that escapes death entering an atavistic reproductive process of polyploidisation and depolyploidisation; it forms the atavistic cyst-like structure aCLS and numerous daughter cells (microcells). The microcell progeny develops a multi-lined cell lineage containing stem cells as well as somatic and reproductive cells and clones. Subsequent aCLSs are formed sequentially by committed daughter cells or occasionally by stressed somatic cells. Accordingly, cancer initiation occurs by genomic changes leading to the amitotic cell state and reactivation of an atavistic life cycle. In humans, atavistic life cycles and hyperpolyploidisation ($n > 16$) are mostly repressed by stable gene regulatory networks – but not in cancer. The permanent UG/MG gene conflict and robust ancient surveillance mechanisms trigger a cascade of molecular lesions leading to genomic heterogeneity and aberrant cancer cell states.

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In memory of Prof. Dr. Ștefan M. Besnea, my great uncle and Head of the Histopathology, Faculty of Medicine, University of Bucharest, Romania, 1923-1940

Introduction

The origin of cancer is not definitively understood. In the past histopathologists understand cancer as a disease of progressive cell dedifferentiation. Later, DNA mutations were thought to be the cause of cancer and cancer was regarded as a genetic disease caused by acquired or parental mutations. The discovery of cancer stem cells later led to the assumption that a deregulated normal human stem cell (hSC) generates spontaneously CSCs that give rise to tumors. More recently, molecular biologists understand cancer as a disease associated with regulatory changes of non coding DNA sequences [1-4] however, it is still not clear whether mutations are causes or rather consequences of cancer. The number of mutated genes identified in cancer increased in the meantime to about 2% of the human genome [5].

Despite the remarkable progress molecular biologists have made, one will not fully understand cancer and its genesis without taking into account the cell biological and developmental aspects of cancer, especially its reproductive life cycle. More research should be done to understand the interconnections between molecular steps and cellular decisions.

Stemness and Life Cycles

Surprisingly, recent studies in protist cell biology have shown that cyclic differentiation by asymmetric cell division are ancient eukaryotic traits [6, 7] and stem & progenitor lineages (SPCLs) originating from the common eukaryotic ancestor, are widely distributed in the eukaryotic world. Lower eukaryotes such as primitive intestinal pathogenic amoebae - best adapted to the human host organism - are capable of polyploidisation-depolyploidisation cycles providing totipotency and stemness to the disseminated microcell progeny [8]. As a result of cyclic processes, cell populations of pathogenic amoebae consist of stem and progenitor cells as well as somatic-vegetative cells changing their genotype when

converting to a pathogenic state which causes liver abscesses [9-12].

In 2007 Erenpreisa and Craig [13] introduced the term *cancer life cycle* as an evolutionary conserved cycle of life, analogous to the life cycles of certain unicellular organisms, supporting the older statements of Sundaram et al. [14] and Rajaraman et al [15] from 2004/2006 that rightly consider stemness as a cyclic property afforded by depolyploidisation of cysts-like structures, later named aCLSs [16-18] or PGCCs [19-23].

As recently shown similarities between the protist life cycle and the development of cancer cell populations and the cancer life cycle are striking [16-18]. Both cancer cells and protists are capable of forming reproductive cysts or cyst like structures (aCLS, PGCCs) by polyploidisation or hyper-polyploidization ($n \geq 8$) and the cyst progeny forms a primary stem cell line capable of differentiating into reproductive and somatic/vegetative cells and clones and sublines. Both in cancer and protists, cysts and aCLSs are formed sequentially by committed daughter cells of the reproductive sublines or occasionally by stressed vegetative cells of the somatic clones. If cancer has really an atavistic origin and performs an ancestral reproductive life cycle, it is conceivable that TP53 mutants are in fact primitive TP53 variants (a/TP53) [24, 25]. Switching to primitive a/TP53 variants such as Ehp53 of amoebae [26] and Dp53 of *Drosophila* [27] could explain why "mutated" TP53 of cancer repairs in a Dp53 manner only DNA damages of the reproductive cells but not the genotoxic DNA damage occurring in irradiated somatic cells.

Aberrant Cell Phenotypes in Tumors

Histopathologists still differentiate between well-differentiated low-grade tumors - early stages of cancer whose cells look *almost normal* - and poorly or undifferentiated tumors (late cancer stages) whose "immature" cells *look very different*. Previous cancer researchers have adopted the idea of reversed differentiation (progressive dedifferentiation) to explain cancer cell phenotypes and thought that tumor cells regress to intermediary stages of non- malignant cell

development [28-30].

However, appropriate research supporting this assumption is missing. While non-malignant cells during differentiation express a given set of genes in a coordinated and repeated template, only a subset of the same gene set is expressed in tumors [31] and this subset can differ from one patient tumor to another as well as between different cells of the same tumor. Differentiation patterns of malignant cells are disorganized. Cancer cell differentiation is *aberrant* and characteristic for tumors [31]. Today it is questionable to assume cancer cells recapitulate in reverse the same developmental stages as non-malignant cells.

Gene Regulatory Networks

Epigenetic Control and Transcriptional Programs of Normal Cells

Historically, the term cell differentiation was introduced in embryology and reflects how embryonic cells controlled by gene regulatory networks (GRN) become specialized in form and function. Cell development occurs by complex gene expression programs highly controlled epigenetically. Gene expression is controlled by specific gene sets that are turned on or off by certain signals inside and outside of the cell. Genes are expressed or repressed and this is what decides how the cell functions. During the multistage process of cell differentiation, the cell changes its ability to respond to different signalling molecules. Signalling molecules are molecules that bring messages to cells, helping them to determine which activities and processes are to be performed [32].

Proteins regulating which genes are transcribed are called transcription factors. There is cellular abundance of expressed transcripts (transcriptome) across the genome. Transcripts appear to be essential to determine the pathway that particular stem cells take as they differentiate. For example, different cell types may arise from the same stem cell population, but divergent transcriptional programs cause them to mature into different cell states. Transcription factors can turn on at different times during cell differentiation. As cells mature and go through different stages, transcription factors can act on gene expression and change the cell in different ways. This change affects the next generation

of daughter cells. Subsequently, it is the combination of different transcription factors that can determine cell type and cell fate [33].

Gene Expression States are Robust and Multi-Stable

One could speak about a gene expression state or a cell state imposed by the GRN dynamics. Each cell state is associated with a distinct gene expression pattern. However, cells can convert from one state to the other. This switching is defined as cell reprogramming. It involves the coordinated changes in the expression status of the genes across the genome. Alterations of regulatory gene expression affect the expression of proteins necessary to implement the new cell state [34]. GRNs are cell state specific and extremely robust to environment changing.

According to Zhou et al. [34] a single GRN produces distinct phenotypic cell states predetermined by a unique genome and each cell state is associated with a distinct expression pattern. Each cell state depends on the gene expression dictated by the GRN that can be modelled as a complex dynamical system. The cell is not free to realize any possible gene expression configuration. The majority of combinations are, in a regulatory sense, not possible and very unlikely to be realized. The degree to which a gene expression pattern is allowed (or not) determines its stability. Cell states appear to be self-stabilizing: similar neighboring states, which are unstable, overtime move to the more stable state (attractor state).

Reprogramming to Embryonic Stemness (iPS)

Cell differentiation is not an irreversible process as previously thought. In the last years it has been shown that almost every cell types may be reprogrammed epigenetically to an embryonic stem cell like state [35]. Already in 2006 the Yamanaka group reset the epigenetic landscape using a handful of transcription factors (Oct4, Sox2, Klf4 and c-Myc) reverting differentiated cells back to pluripotency [36]. Over the course of a few weeks some of the treated cells start to divide faster (fast cycling) and begin to quickly lose their differentiated cell characteristics by robust downregulation of somatic genes. This down regulation meant, the cells were converted to an embryonic stem cell-like transcriptome/epigenome with pluripotent

capabilities [37].

IPS reprogramming is mostly induced by gene overexpression [38-40]. It confirms that epigenetic modifications are dynamic. By reprogramming, genes or small molecules that confer the stability of gene expression are shut off or withdrawn and the manipulated cells maintain the reprogrammed cell state. The persistence of the new cell state is a self-enforcing property and therefore an elementary property of reprogramming [34]. The GRNs that govern cell development have long been suggested to face the trade-off between cell state stability and flexibility (cell plasticity). Intrinsic stimuli or extrinsic signals must be able to trigger the exit from the initial differentiated state and entry into the new cell state. Research highlights the concept of network dynamics and cell reprogramming by well determined sets of genes [34]. One would have to learn more about the biology of the regulatory networks to understand how stable but rare intermediates and rare transdifferentiation subtypes may occur.

Reprogramming of Somatic Cancer Cells to Secondary CSCs

In the past more and more people consider that tumorigenesis is connected with CSCs formation [40] but stem cell formation and their activation is not a process of reprogramming by oncogenic mutation as considered by Wahl and Spike [41] and others [42,43]. However, the question is, are mutations really necessary and sufficient to promote cell phenotype changes during tumor progression? Pisco and Huang [44] have two contra arguments: one is the clonality of cancer cell populations that contains both CSCs and more differentiated somatic cells (co-existing together) and the second is the reversibility of phenotype switching in the tumor cell population [45-47]. Phenotype changing (cancer cell plasticity) is non genetic and not caused by mutations [44]. It leads to the assumption that somatic cancer cells are in fact "facultative stem cells".

More recently the bidirectional switching from somatic cancer cells to new CSCs clones was described as a structural part of the atavistic cancer cell model [17]. It assures changing of genetic and

epigenetic information in cancer cell populations as well as increased resistance by genomic polyploid rearrangements. The assumption of Pisco and Huang's [44] that evolutionary ancient gene expression programs are implied in cancer cell plasticity is subjected by the atavistic cancer cell lineage model proposed by us [16-18]. Cancer is in our cell biological opinion a disease of return to atavistic stem cell lineages and surveillance mechanisms (cancer life cycle).

Cancer Cells are Refractory to Terminal Differentiation

In non-solid cancers such as acute promyelocytic leukemia (APL) but also in naso-pharyngeal carcinoma (NPC) and adenomatous polyposis coli (APC) it was possible to reactivate endogenous differentiation programs in order to eliminate tumor phenotypes and initiate cell maturation (terminal differentiation) [48, 49]. According to the authors exogen factors such as all-trans-retinoic acid (ATRA) in APL arrests myeloid cell maturation at the promyelocytic cell state and disrupts the causative alpha fusion protein. Similarly, restored IKK α kinase in poorly differentiated NPC cells induces terminal differentiation decreasing tumorigenicity. However, most cancer cells cannot revert to normal cells with relevant functionality [48].

Molecular Tumorigenesis

Once it is clear that (i) cells controlled by GRN are not free to realize any possible gene expression configuration and (ii) differentiation patterns of malignant cells and tumors are disorganized and *aberrant* [31], the question is how oncogenic disorders occur and what causes cancer.

There is Significant doubt that Cancer Initiates from Mutations

Are mutations merely consequences or causes of acquired cancer? Mutations are alterations of DNA sequences of genes occurring by mispairings and changes in one DNA base pair. They result either in the substitution of amino acids in the protein made by the gene or in a shortened protein that may function improperly or not at all. Other mutations occur by insertion, deletion or duplication of a piece of DNA. Genetic variations can have large effects in cancer

initiation. On the other hand, certain mutations in the cancer repressor genes BRCA1 or BRCA2 greatly increase the familial risk for either breast or ovarian cancer [50].

Errors are a natural part of replication but usually repair enzymes recognize structural imperfections removing and correcting them. Some replication errors make it past these mechanisms becoming permanent mutations. Spontaneous mutations occur in the absence of stress and environmental damages such as radiation and chemicals. Moreover, when genes for DNA repair enzymes become mutated, mistakes accumulate at a much higher rate [51]. After the next cell division incorrectly paired nucleotides become permanent mutations and served as templates for further replication events. Not all mutations are bad and it is not a direct correlation between mutations and cancer. Some mutations lead to genetic variation and evolution. However, many researchers sustain the idea that somatic mutations accumulated during proliferation and cell division of somatic cells may result in cancer [51, 52] but a growing number of researchers believe that mutations are effects and not causes of cancer. For example, many mutations detected in pancreatic cancer are present in the pancreas of older persons who never develop pancreatic cancer in their lifetime [53].

Epigenetic Gene Silencing is more Predominant than Mutations

Cancer researchers have found that tumors are mosaics of mutant cells containing both genetic and epigenetic changes that distinguish them from normal cells. Mutations and epimutations are heritable: daughter cells inherit by cell division genetic and epigenetic abnormalities of the mother cell and may acquire itself new genetic and epigenetic abnormalities. Epimutations occur via DNA methylation, histone modification and RNA interference [54]. However, many of the genetic and epigenetic abnormalities correspond to increased proliferation rates of mutant populations [55-57]. In the atavistic opinion however, the proliferation rate depends rather on oxygen contents: primitive cell types such as intestinal amoebae are capable to perform oxygenic cell cycles in less of 5-6 hrs [6,7, 12].

System instability is considered to be the major contributing factor of genetic heterogeneity in cancer. In most solid cancers (breast cancers, melanoma, and lung cancer) genome instability comes from the large frequency of mutation in the whole genome DNA sequence [58-60] and from multiple cycles of clonal and non-clonal expansions. The best-understood alterations in tumor cells are the silencing or down regulation of gene expression by changes in the methylation of nucleotides. Methylation changes are thought to occur more frequently than DNA mutations; they are responsible for many changes during tumorigenesis and neoplastic progression. In cancer, loss of expression of genes occurs about 10 times more frequently by transcription silencing than by mutations [61]. Transcriptional silencing may be of more importance than mutation in leading to progression of cancer. In colorectal cancer there are 600-800 genes transcriptionally silenced [61, 62]. Another path to transcriptional repression occurs by altered expression of microRNAs [63].

Multi-Mutations in Cancer: Cancer Genes, Cancer-Driving Proteins

There is a wide spread belief that normal human cells become cancer cells largely because mutations in their genes. On the popular internet websites of many National Cancer Societies [64-65] mean mutations transform normal genes to become "cancer-causing genes" and refer to the genes having mutations linked to cancer as "cancer genes". It is thought that (i) many mutations are needed before a cell becomes a cancer cell and (ii) cancer-causing mutations would accumulate over the course of a life time. On the other side, hereditary cancers are rare and it is believed that people inheriting mutated genes from the parents get the same type of cancer faster as a person getting sporadic cancer by acquiring a multitude of mutations during its own life period. As a consequence, hereditary cancers are more aggressive than the sporadic occurring cancers and do not respond to the typical treatments for sporadic cancer forms.

As reported by Bailey et al. [66] and Li et al. [67] researchers found more than 290 genes for which molecular lesions (mutations) are known. The "cancer genes" represented at the time more than 1%

of the human genome [5]. Many researchers considered that mutated genes are causative in tumorigenesis [68]. Since the first report their number has increased to about 1000. Some mutations prevent a protein from being made, others may change the functionality of proteins and others up-regulate genes to overexpress a protein. Most genome-guided cancer treatments work today by blocking cancer-driving proteins. 31 targeted therapies approved by the FDA (USA) work in a manner similar to Gleevec, a receptor related drug [69]. Other drugs, such as Larotrectinib, target mutant cancer genes such TRK fusion genes [70].

Common Driver Mutations and Passenger Mutations; Specific Subsets of Mutation (Driver Pathways); Tumor Suppressor and DNA Repair Genes

Mutations affect both *tumor suppressor genes* and *DNA repair genes* that control normal cell growth and cell division, and prevent errors in DNA, but tumor suppressor genes may exhibit more frequently altered expression [68]. Despite genetic and phenotypic heterogeneity observed in cancer, most tumors share certain characteristics leading to the idea of *common genetic pathways* that are deregulated in all cancer cells. More often than not, so called *tumor-initiating mutations* may predict what types of mutations occur later in the progression of certain tumors. Such genetic pathways were initially discovered in colon cancers. Each of the histological changes occurring in adenomas evolving to carcinomas is accompanied by the mutation of specific genes [71]. There are subsets of mutations that correlate with specific types of cancer, and subsets of genes that correlate with the degree of malignancy [72-73].

Recently more and more studies distinguish between (i) common driver mutations - which contribute to cancer initiation and development - and (ii) passenger mutations which accumulate in cells but do not contribute to carcinogenesis [66, 74-79]. Presumed common driver mutations - fundamental to the disease process - were identified in over 20 significantly mutated genes. Commonly mutated genes at substantially high mutated frequency are TP53, KRAS, SMAD4 and CDKN2A [53, 80]. The dominant common mutations subject cell-cycle regulation genes (TP53, CDKN2A), DNA damage repair genes (BRCA1, BRCA2, PALB2), DNA

mismatch and other intracellular processes. Many other genes are mutated at substantially lower frequency. Usually, the median number of mutated genes is about 60 per cancer [53]. Persons with mutations in DNA repair genes are likely to acquire additional mutations.

Recently researchers have turned their attention to a group of genes (gene sets) derived from known pathways of protein-protein interactions which may be frequently perturbed within tumor cells and lead to acquisition of carcinogenic properties.

Interrelations among different Mutated Driver Gene Pathways: Cooperativity Instead of Mutual Exclusivity

In the past there were not suitable methods available to detect individual driver genes carrying recurrent mutations [81]. That's why researchers at the time considered driver genes as *high coverage genes* of high mutual exclusivity, although they cover a high number of cancers and tend towards mutual exclusivity. A single mutation - for example the mutation of TP53 - would be usually enough to disturb one pathway. Thus, it was believed that TP53 coverage is exclusive and the p53 pathway does not occur simultaneously with other driver mutations. However, recently researchers discovered a certain *cooperativity* [74, 82-84] existing between distinct mutated driver pathways (MDPs) [57, 85-87]. This cooperativity among different pathways likely occurs simultaneously in a large cohort of patients [88]. It is a co-occurrence of *common MDPs* and *individual-specific MDPs* [74, 78]. Hoadley et al. [89] found similarity among driver gene sets across distinct cancer types. According to Zhang and Zhang [78] there are eight common driver gene sets of BRCA, indicating the complexity of BRCA carcinogenesis.

Recently Marticorena et al. [77] describe universal patterns of selection; which look like driver mutations enabling cancer cells to evade normal constraints on cell proliferation to invade tissues and other organs. The number of mutations driving cancer varies considerably across different cancer types. Some of them occur in genes that are not yet identified as cancer driver genes. They are many more genes remaining to be discovered [90]. The authors propose, there is a relatively small constant number of mutated

genes (N= 2-11) necessary to convert a single normal cell into a cancer cell [77].

Specific Cancer Driver Modules

Previously efforts were made to detect genes with significantly higher mutation rates [81, 91] namely gene mutations that are enough to perturb a relevant genetic pathway [92, 93]. Since it is known that genes with driver mutations work together in regulatory pathways [92, 94] researchers believe that searching driver modules (driver gene sets) will lead to a better understanding of carcinogenesis at the pathway level. As reported, different cancer types have common driver genes (such as TP53), as well as specific driver genes that play different roles in different cancer types. The driver modules detected in a single cancer type always contain common and specific counterparts [67]. There are similarities and differences in the frequency of individual pathway alterations [66]. Detection of cancer specific driver modules (including specific genes), is important to be able to understand the different mechanisms of different cancers at the pathway level [67].

Compared to the huge body of molecular biological data regarding cancer progression and tumorigenesis, there is little data regarding processes by which a normal non-cancerous cell actually transforms into the cancer cell of origin initiating cancer.

What Really Initiates Cancer?

Adjiri [95] published research concerning the role of DNA mutations as both driver and passenger in cancer, highlighting that DNA mutations are contributors for the development of a tumor - once it has initiated. However, according to Adjiri, drivers would not have a role in cancer initiation. The author proposed to give more focus to the events responsible for the switching of a cell from normalcy to malignancy, especially the changes which are taking place at the evolutionary level. The suggestion is that there are highly developed evolutionary constraints that act as a barrier to preserve multi-cellular surveillance mechanisms that prevent cancer. The author is not sure whether going after DNA mutations can one day lead us to inhibit the appearance of cancerous cells. The authors suggest shrinking a tumor is one thing but preventing the genesis of

transformed tumors is a totally different matter.

According to Adjiri [95], the numerous DNA mutations observed in cancerous cells could be regarded as symptoms or consequences of transformation, suggesting that the driver in cancer initiation may not be a particular mutation in DNA that translates into a causative role [95]. Mutations do occur in DNA, but without causing cancer. Aside from the numerous breakthroughs in genome sequencing results, our understanding of cancer as a disease remains poor. The author concludes that the objective in cancer therapy should not be limited to improving the overall survival of cancer patients but rather to cure all cancer patients regardless of the genetic characteristics of their tumors. Cancer may not be primarily a genetic disease, meaning DNA changes would be causal events as described in literature. Cancer could rather be described as a disease with a cause but something still unknown at a cellular level, which reprograms a cell for survival. Adjiri said, the time is ripe to go a step farther and move cancer research in a fundamentally new direction.

Disruption of a Gene's Regulatory Elements

In the last 4-5 years scientists have found that the number of human genes that code for proteins is not as numerous as previously thought and put this number at less than 19000 (1-2% of the whole genome). 98% of the human DNA does not code for proteins. Scientists consider, hidden switches of regulatory elements dial gene expression up and down [4]. There are hundreds of thousands of functional regions (non-coding genome portions) whose task is to control gene expression. Their number is overwhelming: there are about 3 Mio regulatory DNA regions thought to contain some 15 Mio binding sites for regulatory proteins (transcription factors) that control gene expression. On the other side, the "dark matter genome" is thought to contain numerous nonfunctional leftovers from evolutionary history [1].

Cancer biologists believe, certain regulatory elements lie hidden among the non-coding DNA driving normal gene expression, controlling the molecular mechanisms of cancer. Results suggest that disrupting a gene regulatory element has a more drastic impact on cell function than disrupting the gene itself [4]. In the case of a transcription factor there are thousand

genomic sites that affect p53 suppression function [96]. Participants to the ENCODE project consider that 10-20% of the non-coding genome has a function that if disrupted, may be significantly perturb the cell [4]. In the light of these findings Polak et al. [97] consider cancer as a “*regulatory and epigenetic program that is superimposed on a cell and the result is the development of genetic and genomic instability*”

Up Regulation of Ancient Unicellular Genes (UGs) and Down Regulation of Metazoan's Gene Pathways (MGs)

One of the most exciting opinions - alternative to DNA mutation theory - comes 2017 from Australia, regarding the evolutionary origin of genes. The Trigos research group [2] mapped 17,318 human genes to a phylogenetic tree consisting of 16 evolutionary human gene phylostrata representing the major evolutionary innovations and made a transcriptional analysis regarding the age of these genes. Human genes assigned to phylostrata 1-3 date back to unicellular ancestors (UC genes), whereas genes assigned to later phylostrata emerged in multicellular ancestors (MC genes). To investigate how the expression of genes in tumors is related to evolutionary origins, researchers calculated the transcriptome age index (TAI) using RNAseq gene expression from seven tumor types and found that all tumors has consistently lower TAIs than their normal counterparts. They uncovered a close association between evolutionary gene age and expression level in RNA sequencing data. Genes conserved in unicellular organisms (UGs) were strongly up-regulated, whereas genes of metazoan origin (MGs) were inactivated. The coordinated expression of strongly interacting UGs and MGs - as occurred in primitive animals - was lost in tumors. According to the authors, 12 highly connected genes controlling UG/MG cooperation are the most important drivers of tumorigenesis.

In the last decade, cancer has been suggested to result from an atavistic process whereby the activation of primitive highly conserved programs lead to molecular phenotypes and population dynamics similar to unicellular organisms [56, 98-100]. Similarly it was suggested that the expression of highly conserved genes is a feature of drug resistance in tumor cells [101]. Trigos et al. [2] show patterns of

co-expression between highly inter-connected cellular processes that are disrupted in tumors. The findings suggest that deeper understanding of the differences in the expression and regulation of ancient UC genes and more recently evolved MC genes will be crucial for uncovering the molecular basis of cancer initiation and providing of new therapeutic strategies.

Life Cycles in Cancer and Protists

Noone has observed directly the cell taking the first step towards oncogenesis, noone knows exactly if the cancer initiating cell is a healthy or an already sick cell, what it lacks, and why it converts to oncogenic transformation. What is known about cancer initiation originates indirectly by observation of already established cancer cells and late tumor cells. This lack of knowledge about the initial step is why we should turn our attention to analogous cell systems occurring in primitive eukaryotes, to understand how primitive cell systems - including cancer - arise and evolve. We propose and assume that the life cycle mechanisms are the same in cancer initiation and development.

A few years ago, we discovered stemness in protists and found that the life cycle of highly reduced eukaryotic cells such as intestinal pathogenic amoebae conserve an ancient multi-lined stem and progenitor cell lineage (SPCL) inherited from the eukaryotic common ancestor. Stem cells were produced by the disseminating microcell progeny hatching cysts [6].

We believe that an archaic primitive lineage and its corresponding gene module(s) is/are conserved in the human genome. In our opinion the initiation of cancer is the reactivation and expression of the atavistic silenced gene module(s). Analogous to protist cysts, cancer forms polyploid cyst-like structures (aCLSs or PGCCs) whose role in the past was poorly understood. We believe that the better understanding of ancient cell lineages conserved in protists may be helpful to understand the mechanisms of cancer initiation.

Amoebae

Hypoxic Stem Cells, Environmental Oxygen and Autonomous Cyclic Differentiation (ACD)

Briefly described, the life cycle of protists begins in the small intestine with cysts hatching and microcell dissemination [6]. The eight totipotent microcells

progress to the colon giving rise to a primary cell line of undifferentiated stem cells. Amoebic stem cells start in the colon two antagonistic sublines, depending of the intestinal oxygen gradient (0.1 to 6.0% O₂ content) or the oxygen content of the resident niche [6, 7, 102]. Stem cells reaching more oxygenated capillary zones convert to reproductive ACD⁺ clones (progenitor sublines) that forms ACD cysts by asymmetric cell division and cyclic differentiation; in more hypoxic zones stem cells convert to somatic- vegetative clones, that also divide by asymmetric division but do not generate cysts (ACD⁻ subline). In cultures of changing oxygen levels hypoxic proliferating stem cells convert to ACD⁺ and ACD⁻ sublines, depending on the O₂ content. Increased hypoxia converts the hypoxic stem cell line to the ACD⁻ subline, while oxygenation favors stem cell conversion to oxygenic ACD⁺ sublines, fast cell cycling and cyclic differentiation. By increasing hypoxia the process of differentiation to cysts is delayed and the oxygenic ACD⁺ subline converts finally into a ACD⁻ subline. Environmental oxygen content is the pivotal driver of stem cell conversion, proliferation and differentiation.

Asymmetric Proliferation and Mitotic Arrested Somatic Cells (MAS cells)

Daughter cells produced in cultures by asymmetric cell division are non-identical. In the case of the reproductive ACD⁺ subline, one of two daughter cells is the self-renewing cell and the second is the committed precursor cell that exits cell cycle by the G₁/G₀ checkpoint; it enters polyploidisation and differentiation (cyst formation). In contrast, the vegetative subline ACD⁻ does not commit the second daughter cell to differentiation; the non-committed daughter cell arrests in a pre-differentiated state of G₀/G₁. These pre-differentiated MAS cells produced by the somatic ACD⁻ subline are the counterpart of committed precursor cells produced by the ACD⁺ subline. MAS cells may reenter the mitotic cell cycle or differentiate to cysts under conditions of stress and nutrient depletion. Switching from one cell state into the other cell state occurs by epigenetic reprogramming, not by mutations. In the course of the disease (amoebiasis), early somatic clones change to more invasive and virulent genotypes capable of invading the liver and other organs [16-18].

Differentiation Potential and Differentiation Switch (DS)

We believe that the decision of whether a cell commits for differentiation or not, or becomes a self-renewing pre-differentiated cell (such as MAS cells) depends on a molecular switches. We assume that a differentiation switch (DS) decides whether the second cell produced by asymmetric division becomes a MAS cell or commits for final cyst differentiation. The molecular DS switch regulates the cell fate to mitotic proliferation (DS/OFF) or differentiation (DS/ON). Differentiation commitment means DS/ON, proliferation DS/OFF. In the process of cyclic differentiation the regulatory switch is always open (DS/ON) while in the case of induced differentiation of MAS cells it must change from DS/OFF to DS/ON. Somatic MAS cells express their *hidden differentiation potential* by opening the regulatory DS switch.

Maybe that Eh/P53 (the p53 variant of amoebae) [26] has a pivotal role in commitment and differentiation of amoebic cells that derive from the early G₁ or G₁/G₀. Stressed MAS cells, in a state of G₂/M, finish first mitotic cell cycle and form committed daughter cells (two G₁ cells) that differentiate post-mitotically to cysts.

In summary, amoebic cells show: (1) all cells have differentiation potential: ACD⁺ cells express the differentiation potential autonomously while ACD⁻ cells express the hidden differentiation potential only by stress; (2) differentiation always occurs from a state of G₁; (3) under conditions of stress a mitotic blocked cell (such as the MAS cell) escapes cell death bypassing to an amitotic reproductive solution; it forms multiple microcell progeny by polyploidisation and depolyploidisation; (4) most of the individual cell states may turn into each other; (5) switching from one amoebic cell state to the other, including the transition from the stressed MAS cells to the reproductive process of polyploidisation and depolyploidisation, is not mutational but epigenetically accomplished.

Cancer

Cancer's Life Cycle and its Atavistic Stem Cell Family

The life cycle of cancer is quite similar. In recent

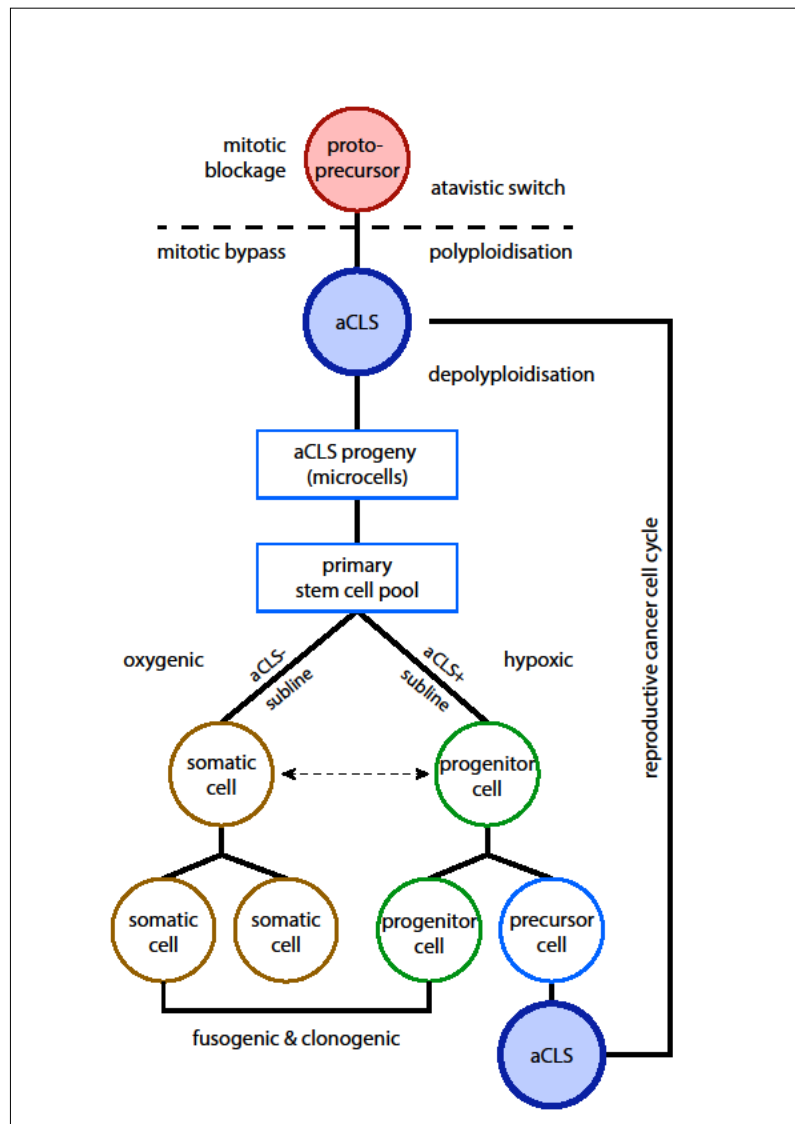


Figure 1. The reproductive life cycle as occurring in cancer and protists: ancient cell lineage, stem cell family and primary stem cells; doi: 10.15406/mojtr.2018.01.00015 [17].

Red is the dysregulated mitotic blocked cell (protoprecursor, cell of origin of cancer). It is in a large sense analogous to the MAS cell of amoebae. It escapes cell death exiting cell cycle bypass and differentiates to the aCLS initiating cancer (blue). Its progeny consists of multiple undifferentiated microcells. Microcells are totipotent, they have stemness potential and form the primary stem cell line. Primary stem cells are the "grandchildren" of the dysregulated protoprecursor; Right is cancer's reproductive subline aCLS+ producing numerous aCLSs (PGCCs) by asymmetric division and cyclic differentiation. Multiple generations of aCLSs give rise again and again to new stem cell lines. Left is cancer's somatic-vegetative subline aCLS-, analogous to the protist subline ACD-. Somatic-vegetative sublines may express hidden differentiation potential in conditions of stress forming new aCLSs and new stem cell lines. Somatic cells are "facultative" stem cells. The individual cell states belonging to the cancer life cycle (self-renewing progenitor cells and committed precursor cells) form in fact an atavistic stem cell family. A molecular differentiation switch (DS) decides if cells of the atavistic cancer family become self-renewing or differentiating.

years more researchers have tried to clarify the relationship between hyperpolyploidy ($n > 16$) and cancer [19-23, 103-104] however, the key role of PGCCs (aCLSs) in starting cancer's reproductive life cycle was little understood. We suppose that initiation of cancer starts from a genomic dysregulated cell blocked in G1 or G0/G1 that is in a certain sense analogous with the MAS cell of amoebae. This dysregulated human cell is capable of reactivating the atavistic gene module initiating the atavistic life cycle of cancer.

In the recent years cancer researchers have experimentally induced polyploid giant cancer cells (PGCCs, aCLSs) by irradiation and chemotherapeutics [19-23, 103-104]. This is not *de novo* initiation of cancer from normal human cells but induced PGCC differentiation from cancer stem cells resistant to irradiation or chemotherapeutics. Stressed induced siCLS, and genotoxic induced giCLS start either from the primary stem cell pool or from reproductive aCLS+ clones (~ 1-2% of the treated cell population) and not from the somatic aCLS- subline (~ 98% of cells). We suppose that some of the self-renewing progenitor cells or committed precursor cells (Figure 1) may repair DNA damage caused by irradiation, likely with the help of an atavistic a/p53 variant. a/p53 variants such as Ehp53 or Dp53 [26, 27] cannot repair DNA damage of the somatic aCLS- subline. Reproductive cells and stem cells after DNA repair form giCLS that give rise to new resistant stem cells capable of metastasis [103].

Proliferating somatic cancer cells are "facultative" stem cells. Unfavorable growth conditions and environmental stress reprogram some of them to secondary stem cells (cell plasticity). There is a lively exchange of information between somatic and reproductive sublines, with or without aCLS formation (Figure 1). On the other hand somatic cells change genotype forming numerous aberrant phenotypes. Reprogramming events are cell-state changes not caused by mutations but many mutations occur as effects of the aberrant phenotypes. We suppose that most of the aberrant phenotypes occurring in tumorigenesis are consequences of the great genetic control conflict that occurs between up regulated UG genes and down regulated MG genes.

Concluding Remarks

Using the analogy with ancient reproductive life cycles and primitive protist SPCL lineages, we consider that cancer is triggered from a dysregulated mitotic cell blocked in a state of G1 or G0/G1 [6,7,12]. Losing its capacity to generate daughter cells by mitosis, the protoprecursor (cell of origin of cancer) reactivates the dark genome gene module of the ancient reproductive life cycle conserved in the human genome. It forms multiple cell progeny by atavistic hyper- polyploidisation and depolyploidisation. We believe that cancer initiation has two distinct phases. In the first phase (*early initiation phase*) the protoprecursor loses early-response genes such as human VRK1 or immediate-early genes such as MYC or FOS that stop its cell cycle causing the mitotic block [105]. VRK1 plays a role in maintaining the reading state of p53. It phosphorylates p53 specifically before passing the restriction point RP (early G1 state). Kinases like VRK1 are also necessary for exiting the G0 state. We suppose that further genomic changes such as inactivation of several life-cycle repressor genes (LCR genes) are needed to activate the silent gene module. The early initiation phase is probably a mutational phase. In contrast, the *last initiation phase* of cancer is not mutational. It is directed by the atavistic gene module. We believe that the loss of LCR genes is the cause of the silent gene module activation.

In this way the defective mitotically blocked cell switches into an ancient level of organization controlled by GRN subcircuits of great antiquity and stable surveillance [106]. The subsequent development of cancer is an intracellular competition between a reactivated gene module of ancestral origin and the rest of the human genome. Atavistic UG genes become dominant subordinating multiple MG genes, which have less and less input. As a result cancer cell phenotypes become more and more aberrant. The human genome does not have effective defense mechanisms against the atavistic aggressor and its surveillance mechanisms. The cancer cell system evolves as an intracellular and extracellular parasite system overrunning its host. The UG/MG gene conflict leads to numerous DNA lesions and mutations. We believe that UC genes can be considered the true driver genes in cancer initiation and development. Genes controlling the switch into the

reproductive life cycle and hyper-polyploidisation (RLC genes) require increased attention from molecular biologists in order to deactivate them. We suppose that such atavistic genes - conserved in the human genome - are evolutionarily related to RLC genes of amoebae that control MAS cells entry into the reproductive life cycle. Comparative molecular biological studies would be useful.

We showed that pre-carcinogenic CSCs are directly related with the cell of origin (protoprecursor) [16-18]. The protoprecursor is the "great-grandmother" of the primary stem cells that are the "great-grandsons" of the cell of origin.

In our opinion similarities between primary stem cells and hSCs are evolutionary conditioned and not directly related. Just like to the modern day protists, hSC have taken ancestral characteristics of asymmetric cell division and stemness from the common eukaryotic ancestor. Common features of early and late cancer stem cells such as migratory capacity, invasiveness, apoptotic resistance, long life span and phenotypic/genotypic changes in the course of the disease are to a large extent common to the pathogen protists invading intestinal tissue and liver.

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