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The reproductive life cycle of cancer: Hypotheses of cell of origin, TP53 drivers and stem cell conversions in the light of the atavistic cancer cell theory

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ABSTRACT

Polyploid giant cancer cells (PGCCs) found in different solid cancers are reproductive cyst-like structures surrounded by an actin envelop. They give rise by hyper-polyploidisation to numerous progeny (microcells, neotic cells) that start a primitive multi-lined lineage and generate subsequent PGCCs by asymmetric cell division and cyclic differentiation. This cancer cell life cycle has multiple similarities with the life cycle of lower eukaryotes (protists) substantiating the atavistic theory of cancer. The primitive cancer life cycle contains several cell types including primary cancer stem cells, somatic cells, as well as reproductive cells, that differentiate new atavistic cyst like structures (aCLSs, PGCCs). Accordingly, cancer stem cells are not transformed normal stem cells (hSCs). Similarities between CSCs and normal hSCs arise from the evolutionary common origin of primitive eukaryotes and more highly evolved eukaryotic cells (stemness evolution). The cell of origin of cancer, as postulated here is a deregulated human cell that has lost, not only relevant control mechanisms and mitotic capacity, but also its normal human p53 network becoming useless for the atavistic life cycle. We believe that this protoprecursor of cancer reactivates an ancient primitive TP53 network originating from the common eukaryotic ancestor. This atavistic p53 helpes to repair genotoxic DNA damages of reproductive cancer cells including CSCs but not DNA damages of somatic cancer cells exposed to genotoxic stress.

Common cancer theories

Until recently, two theories dominated the carcinogenetic discussion. One is the stochastic theory which supposed that the transformation of a normal cell into a cancer cell depends on the sufficient accumulation of mutations in the cell of origin. The other is the CSC theory which supposes that tumors arise from defective human stem cells (hSC) which then form cancer stem cells (CSC). Both theories have weak points, as they mostly rely on assumptions and less substantiated findings. Many researchers today doubt that accumulated mutations are the cause and are instead effects of carcinogenesis; stem cells transformation to CSCs has never been observed in laboratories.

In common theories, the cell of origin of cancer (initiating cell) goes through a process of transformation and deregulation, that occurs by mutations of genes and epigenetic changes [1,2]. Many researchers suggest that oncogenes may cancel tumor suppressor mechanisms; by this way transformed cancer cell comes out of control. Accordingly, malignant transformation occurs by multiple genetic/epigenetic alterations not limited to a unique cell generation. One speaks of early stages and late stages of transformation respectively progressive acquisition of malignancy [3]. As a result of transformation cells lost their lineage commitment potential and form tumors.

Atavistic cancer theory

In the last years there are more and more data favouring the atavistic cancer theory. Cancer researchers found that tumors are mosaics of cells containing not only genetic but also *epigenetic changes* [4–6]. The best-understood epigenetic alterations are the silencing of expression of genes by changes in the methylation of nucleotides. Methylation changes are thought to occur more frequently that DNA mutations, being responsible for many changes during tumorigenesis and neoplastic progression. According to Vogelstein et al. [7] loss of expression of genes occurs about 10 times more frequently by transcription silencing than by mutations. Transcriptional silencing may be of more importance than mutation in cancer progression. In colorectal cancer

Abbreviations: aCLS, atavistic cyst like structure; PGCC, polyploid giant cancer cell; CSC, cancer stem cell; hSC, normal human stem cell; ACD, autonomous cyst differentiation; UGs, unicellular genes; MGs, multicellular genes; siCLS, stress induced CLS; giCLS, genotoxically induced CLS

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there are 600–800 genes transcriptionally silenced [7,8]. There are the geneticist J.A.Stamatoyannopoulos of the University of Washington in Seattle [9] that considers cancer as a *"regulatory and epigenetic program that is superimposed on a cell and the result is the development of genetic and genomic instability"* [9a].

Even 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 features and population dynamics similar to unicellular organisms [10–13]. Similarly it was suggested that the expression of highly conserved genes is a feature of drug resistance in tumor cells [14]. One of the most exciting opinion – as an alternative to DNA mutations – comes 2017 from Australia, regarding the evolutionary origin of genes. Anna Trigos et al. [15] investigate how the expression of genes in tumors is related to the evolutionary origins. They uncover a close association between evolutionary gene age and expression level in RNA sequencing data. Genes of unicellular origin conserved in the human genome (UGs) were strongly up-regulated, whereas genes of metazoan origin (MGs) were primarily inactivated. The coordinated expression of strongly interacting UGs and MGs – as occurred in primitive multi-celled organisms (primitive animals)– was lost in tumors. According to the authors 12 highly connected genes controlling mediate UG/MG cooperation are important general drivers of tumorigenesis.

In 2017 Adjiri [16] sustain 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 talking place at the evolutionary level. The author is not sure whether going after DNA mutations can one day lead us to inhibit the appearance of cancerous cells. Shrinking a tumor is one thing but preventing the genesis of transformed cells is a totally different matter. According to Adjiri the numerous DNA mutations observed in cancerous cells could be regarded as symptoms or consequences of cell transformation, suggesting that the driver in cancer initiation may not be a particular mutation in DNA that translates into a causative role. Cancer may not be primarily a genetic disease, meaning DNA changes would be causal events as described in the previous 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.

In recent years more researchers tried to clarify the relationship between hyperpolyploidy and cancer [17–23] however, the key role of the polyploid giant cancer cells PGCCs in cancer initiation and starting the reproductive cancer life cycle was not completely understood. PGCCs are hyper-polyploid atavistic cyst like structure (aCLS) with an envelope of actin found in several solid cancers [2]. They give rise to multiple microcell progeny that disseminate. The process of aCLS formation and microcell dissemination was described by Sundaram et al. [25] and Rajamaran et al. [26] and was named neosis. According to Rajamaran neotic cell formation is a cyclic event and neotic cells are linked to the process of stem cell formation and tumorigenesis. Erenpreisa and Cragg [27] describe stemness as a transient, cyclic property afforded by de-polyploidisation and attempt to reconcile this view by the idea of a *cancer life cycle* analogous to the life cycles of certain unicellular organisms. Accordingly, reproductive polyploid cycles and aCLS progeny ensure cell system survival in protists and cancer by totipotency and stemness recovery [28]. Studying the biology of intestinal pathogenic protists we recognize the primitive stem cell lineage hidden in the life cycle of protists as a universal eukaryotic model [29,30].

We see a great analogy between the reproductive life cycle of protists and cancer's life cycle. Both systems start from a defective cell (mebolically stressed cell) blocked in a state of G1 [30–32]. This cell loses its capacity to produce new daughter cells. It seeks for a reproductive solution and finds it conserved in the own genome. Metazoans and humans conserve an ancestral gene module (UG gene set) capable of reactivating an ancient reproductive life cycle and stem cell lineage that is wide spread in the world of single celled eukaryotes

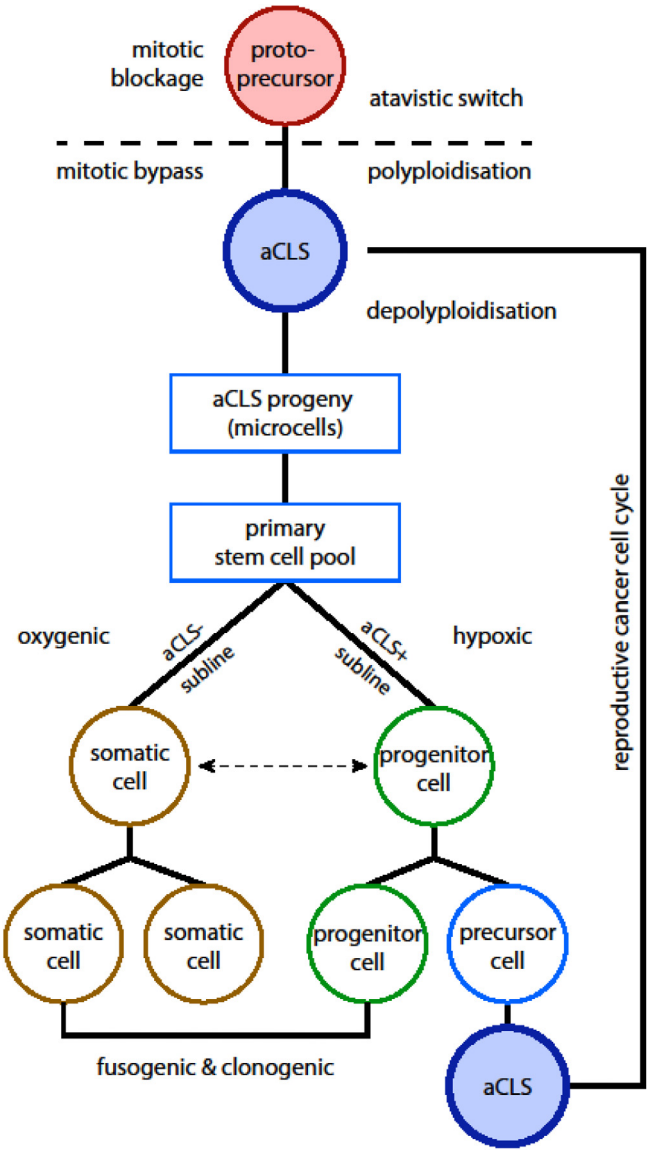

Fig. 1. Cancer initiation and cancer life cycle. A human mitotic blocked cell (protoprecursor) escapes imminent cell death and reactivates an atavistic stem/progenitor cell lineage that bypasses mitosis and starts the reproductive cancer cell cycle, analogous to metabolically stressed protist cells (from doi: 10.15406/mojtr.2018.01.00015).

(protists). This wide spread reproductive life cycle – inherited from the common ancestor – gives rise in humans and animals to tumors.

We believe that carcinogenesis is an evolutionary regression and return to an atavistic process of cyclic hyper-polyploidisation (N > 8) and cyst like formation, unknown to mammalians and human cells. Once initiated the reproductive cancer cell cycle does not stop. It leads to a parasitic system that evolved continuously on the cost of the host organism that does not posses sufficient defense mechanisms against the aggressor and can not prevent its proliferation.

The cancer life cycle model

In the light of our primitive life cycle model (Fig. 1) [24,29,33] cancer cell populations consist of three interrelated subpopulations that are produced by distinct cell lines/sublines: (i) somatic cancer cells, (ii) atavistic reproductive cancer cells and (iii) cancer stem cells. Carcinogenesis begins from a deregulated mitotic blocked cell (protoprecursor or cell-of-origin) that bypasses mitosis forming an atavistic cyst like

structure (aCLS, PGCC). The proliferating blocked cell bypassing mitosis is evolutionary analogous to metabolically stressed protists that enter reproductive polyploidisation-depolyploidisation cycles (encystment) under stress conditions. The microcell progeny of the aCLS generates the primary stem cell line that differentiates – in an environment dependent manner – to form two antagonistic sublines: a reproductive more hypoxic cancer cell subline capable to form further aCLSs by cyclic differentiation (aCLS + subline) and a somatic more oxygenic subline that proliferates cyst-free (aCLS − subline). The somatic subline maintains differentiation potential forming in conditions of stress siCLSs (stress induced cyst-like structures) or giCLSs (genotoxic induced cyst-like structures) [24,33]. Both aCLS + and aCLS − sublines are analogous with the ACD + and ACD − sublines of protists [29]. We introduce the enlarged term of cancer stem cell family for reproductive and somatic cancer cells capable to form aCLSs, siCLS, giCLS and thereby multiple CSCs generations [33]. In contrast to our life cycle model, some other atavistic models subject elements of previous cancer models considered not mutually exclusive: the clonal model, the hierarchy model and the plasticity model [34].

Self renewing cells of both aCLS + and aCLS − sublines are capable of cell fusion and gain of function processes (GOF). They give rise to *hybrid cancer cells* of new phenotypes, genotypes and tumorigenic potential. Environmental cues reinforce clone development. Genotype changing also occurs in primitive eukaryotes such as intestinal amoebae that invade liver by high invasive clones. In the following we highlight the validity of the atavistic model as compared to the other cancer models.

Primitive p53 gene networks in cancer and protists

In the theories so far the prerequisite for the cancer initiation and development is considered to be the loss of p53 wild-type function (WT p53) that is replaced by WT p53 mutations. Cancer researchers consider the human p53 network as “the guardian of the genomes” playing a crucial role to suppress genomic instability that drives cancer progression; they assume that the loss of WT p53 function promotes carcinogenesis by enabling accumulation of genetic mutations in human cancer cells [35]. As a guardian of the genome p53 would prevent the accumulation of genetic mutations inducing cell cycle arrest, apoptosis or senescence [35]. It is still an open question whether mutations of the TP53 gene are involved in the initiation of malignant transformation or perhaps only at more advanced stages of cancer. Rivlin et al. [36] consider that mutations in the p53 gene occur to different late phases of the multistep process of malignancy, thus contributing differentially to tumor initiation, promotion, recurrence and metastasis.

The question is: (i) does the protoprecursor also reactivate ancestral p53 genes, and (ii) is the human p53 network in cancer cells “mutated” or replaced by an ancestral network? TP53 networks have a long evolutionary history. Ancestor genes are found in single celled eukaryotes and early metazoans closely related to a p63/p73-like hybrid gene [37,38]. During protist evolution, the ancestral hybrid genes duplicate to a gene most closely related to late TP53 genes. Interestingly, the function of the ancestor gene is to protect *germ-line cells* from DNA damages, watching over of the genome fidelity of descendants. According to [38] the earliest functions for the p53 family are used in conditions of stress signals to ensure germ-line’s genome integrity. Highly evolved TP53 gene focuses upon the duplication of stem cells, progenitor cells and somatic cells to prevent aberrations by DNA replication.

Modern day protists such as pathogenic intestinal protists possess the Eh/Ei-p53 gene that is oldest known evolutionary ancestor of the mammalian TP53 gene. Questions of the functions of Eh/Ei-p53 are open however, Eh/Ei-p53 protein levels increase in irradiated amoebae [39]. In the last years it is evidence that an ancestral focus of p53 action could operate in primitive stem cells, indicating an ancient intimate link between p53 and stem cell biology. The pivotal role of the TP53 gene family as tumor suppressors took place later by evolution to vertebrate lineages [40]. In non-vertebrates and short living metazoa (Drosophila) only the stem cell compartment is licensed for stress-induced activation of p53 regulatory networks. As highlighted by *eLife digest* [40], protists and primitive metazoa have special mechanisms to protect stem cells from DNA damage: damaging DNA activates ancient p53 in stem cells and their progeny, but not in the other cell types that have also been damaged. Similar effects were observed in cancer where genotoxic agents affect only somatic but not reproductive cancer cells. We suppose that this archaic p53 focus (a/p53) operates not only in ancestral stem/progenitor cell lineages of protists but also in cancers controlling recurrence and chemoresistance.

From the cell of origin to early pre-carcinogenic CSCs

In the classic understanding the human cell that receives the first cancer-causing modification is the *cell-of-origin of cancer* also called cancer-initiating-cell or precursor of cancer, while cells inducing tumors or maintaining tumor propagation are *cancer stem cells* (CSCs) also named tumor-initiating- cells or tumorigenic cells. In 2006 the American Association of Cancer Research considered CSCs as self renewing cells within a tumor capable to cause the heterogenous lineages of cancer cells that comprise the organism [34,41]. In current theories the relationship between the cell-of-origin of cancer and CSCs are not well understood [42]. Our atavistic model (Fig. 1) solves the uncertainty. We showed that pre-carcinogenic CSCs are directly related with the cell-of-origin (protoprecursor) [33]. The deregulated protoprecursor starts the reproductive cancer cell cycle via hyper-polyploidisation and aCLS differentiation, and the disseminating microcell progeny of aCLSs forms the primary stem cell line; in turn primary stem cells generate the reproductive aCLS + subline forming new aCLSs. In other words the protoprecursor is the “great-grandmother” of the CSCs, and CSCs are the “great-grandsons“ of the cell or origin [33]. Later in development aCLSs are cyclically produced by the aCLS + subline.

It is significant that a group of researchers at the University Libre of Bruxelles (ULB), in 2016, found that cells-of-origin control the late malignant EMT transition; they consider that EMT genes are primed in the cancer cell-of-origin, that facilitate malignant development [43]. Thus, the cell-of-origin controls tumorigenic processes and EMT related heterogeneity. It has wide transcriptional and epigenetic capacities for cancer development.

In our opinion CSCs do not originate from a transformed human stem cell (hSC). Similarities between CSCs and hSCs are evolutionary conditioned. Just like to the modern day protists, hSC have taken over ancestral characteristics of asymmetric cell division and cell differentiation (stemness). Common features of early and late CSCs 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 [32]. Under the effects of the gastro-intestinal microenvironment protists also change genetically/epigenetically profile, forming clones of increased hepatic virulence [44,45].

From early pre-carcinogenic CSCs to tumorigenic CSCs

Similar to amoebic intestinal development, cancer cells have a two way relation to their microenvironment: the microenvironment does affect cancer cells and cancer cells may change its microenvironment [44–49]. We agree with the idea of Valle et al. [50] that tumorigenic CSCs are highly plastic and have multiple properties acquired by clonal evolution. In the atavistic model we assume that self-renewing cells of both somatic and reproductive sublines are capable of phenotypic, epigenetic and genotypic changing, inheriting the gained characteristics to CSCs via atavistic cysts like structures (aCLSs, PGCCs) and their microcell progeny [33]. Our model is in accordance with the studies cited above that assume that tumors may contain genetic/epigenetic

different CSC subclones capable of metastatic dissemination. Furthermore, we agree with the idea that cancer stem cells and cancer somatic cells are in fact flexible "cell states" of the same cell lineage, capable to increase continuously their heterogeneity and invasiveness potential [33] as observed in protists [44,45]. As mentioned by Valle et al. [50] stem like phenotypes are intimately linked to CSC resistance and factors involved in CSC chemoresistance can be regulated epigenetically or by miRNA [51,52].

Concluding remarks

While previous cancer models and theories leave open a lot of questions about cancer initiation and development, the atavistic life cycle model has more relevant and competent answers. Thus, it is evidence that precarcinogenic CSCs do not originate from a directly transformed human stem cells hSCs; they are descendants of the deregulated protoprecursor (cell-of-origin of cancer). We consider the atavistic cancer cell lineage as an enlarged stem cell family: all cells (reproductive or somatic cancer cells) maintain aCLS differentiation potential and may produce by this way new CSCs. Somatic cells expressed its hidden differentiation potential in conditions of stress or genotoxic stress forming siCLSs or giCLS and CSC capable of EMT. In our opinion the protoprecursor cell change the human p53/WT network (hp53) by the atavistic p53 network (ap53). This exchange was termed in the past as "p53 mutants" (mu-p53). In contrast to hp53, ap53 repairs only DNA damages of reproductive cells and CSCs but not DNA damages of aggressed somatic cells. Genotoxic damages lead somatic cancer cells into cell death. In other words, ap53 repairs damaged CSCs selecting finally an invasive CSC population of high tumorigenic and metastic potential. All the considerations above open new avenues for research in cancer.

Conflict of interest

None.

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