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Review Article

The Ancient Biological Principle of the Retrograde Cellular Immortalization -

Joseph G. Sinkovics*

Department of Molecular Medicine, The University of South Florida Morsani College of Medicine; Cancer Institute, St. Joseph's Hospital, Tampa, FL, USA

***Address for Correspondence:** Joseph G. Sinkovics, Department of Molecular Medicine, The University of South Florida Morsani College of Medicine; Cancer Institute, St. Joseph's Hospital, Tampa, FL, USA, Tel: +813-870-4255; E-mail: Sinkovi.Joseph@baycare.org

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ABSTRACT

The primordial RNA/DNA complex possessed an inherent faculty for the protection of the cellular life forms from death due to exposure to physicochemical and biological intrusions. The proof for it is the survival and the close to the limitless expansion of the cellular life forms, as casted against the violent history of this planet. A long list of elementary pathways was used alternatingly by the primordial cellular life forms for the ontogenesis of their progeny, acquisition of their immune defenses, and their survival in grave distress. The ancient faculties were preserved and further evolved when diploblastic life forms have become large bilaterally organized triploblastic multicellular communities (including Homo). The original ultimate bioengineer RNA/DNA complex preserved and further evolved its ancient faculty for exemption from senescence, by duplicating in young age (a semblance of immortality), especially in unicellular life forms. In single cells of multicellular eukaryotic life forms, these inherent capabilities dominantly involve anti-apoptosis, autophagy with recovery, and somatic driver mutations, all of these resulting in accelerated mitoses. These primordial faculties may be re-activated and re-expressed in bilateral triploblastic macro-organisms occupying top positions in the evolutionary ladder (Homo). In these hosts, these faculties were placed under the control of an extensive inhibitory network. The origin of such a network is already detectable in the dictyostelia amoeba in the form of the retinoblastoma protein regulating the cell divisions of its host.

This article distinguishes between those reactive processes, which were induced by external physicochemical and biological initiators, and thus evoke strong defensive reactions (which often may fail), and those that were internally commenced in some selected single cells of multicellular hosts by ancient retroviral genomic inserts incorporated into the recipient genomes. These latter processes are capable of switching off all specifically directed immune defenses and even turn them into stimulatory forces within the internally transformed cells. In addition, these transformed cells receive full biochemical support that their hosts render to them via fibroblasts and macrophages, so altered already in the initially shared tumor/host microenvironments. This faculty inherently inscribed in the original RNA/DNA complecti and recurrent in highly evolved life forms is hereby referred to as 'retrograde cellular immortalization'. It is clinically diagnosed as oncogenesis.

INTRODUCTION

Carl Woese, et al. [1] saw in the archaea the first cells living on the ancient Earth. The extraordinary laboratory efforts toward constructing a self-metabolizing single cellular structure that multiplies, so far have failed (cited here is the popular assessment of Michael Marshall http://www.bbe.com/earth/story/2016. Basic elements of the envisioned RNA World could not be reproduced in the laboratory: in test-tubes the sugars did not unite promptly with the nucleotides; RNA molecules failed to readily replicate in the absence of the enzymes that synthesize these molecules on templates. The evolution of cellular life on the ancient Earth is explained at a higher level by S. D. Domagal-Goldman and Katherine E. Wright, et al. [2], co-lead editors in the Astrobiology Primer v2,0. The natural origins of the RNA/DNA complex and its inherent faculties just now are becoming reproducible in the protocells constructed in the laboratories of John Sutherland, et al. [3,4], and Jack Szostak, et al. [5-8]. On the primordial Earth, the nucleic acid precursors, Hydrogen Cyanide (HCN) and Hydrogen Sulfide (H_2S) formed RNA nucleotides in UV light. Acetylene and formaldehyde could have produced the four RNA nucleotides [3]. Non-enzymatic template-directed RNA - replication must have pre-dated the activities of the ribozymes [4]. Prior to the appearance of the ribozymes, 5'-activated oligonucleotides (oligomers) catalyzed the primer extension products in sequential additions of activated monomers, so much so that a hammerhead ribozyme structure was taking shape. 2-Aminoimidazole-activated nucleotides accelerated this procedure [5,6]. Non-enzymatic copying of RNA templates occurred in the RNA world. In the laboratory, it was possible to bind ribonucleotide monomers to RNA primer-template complexes, a form of template-directed non-enzymatic RNA replication. A synthesized un-reactive phosphonate-linked pyrazole analogue of the highly activated nucleotide, guanosine 5'-phosphoro-2-methylimidazolide carried out non-enzymatic primer extensions in Watson-Crick base pairing, that is guanine-cytidine base-pairs. The resulting structures were in disorder. The distances between O_3' of the primer and the P atom of the monomer were too long at 4.5 to 6.5 Å, therefore non-reactive [7] (see all images including three-dimensional X-ray crystallography; free text). Thus, disorderly non-canonical base-pairing occurred in the pre-biotic world. Some self-splicing introns preserved non-canonical base-pairing (cited are those in the unicellular eukaryote *Tetrahymena thermophila* and the archaea, *Haloarcula marismortui* [7]. Depicted is the synthesized and activated 3'-amino-3'-deoxy-2-thio-thymidine, as a substrate for non-enzymatic copying of nucleic acid templates [8]. The primordial high affinity RNA-protein cross-linking between isotope-labeled oligonucleotides and covalent peptides with oxygen-labeled phosphodiester linkages in the oligonucleotides, have been accomplished in the laboratory, using lin-28 and let-7 pre-element microRNAs [9]. The conserved derivatives of the miR lin (lin28) isoforms have become tumor suppressors, while those of miR let (let7) act as proto-oncogene promoters in the human genome [10]. Occasionally, template directed non-enzymatic replication of RNA resulted in a double-stranded product. Separated strands could re-anneal. Oligoarginine peptides acted as inhibitors to the annealing of complementary oligoribonucleotides [11]. In contrast, the much later appearing ribozyme polymerases enforce accurate Watson-Crick pairing of the incoming monomers to the recipient templates, but the time and circumstances of the epoch-making biological switch has not been as yet discovered. A pre-cellular ribozyme-armed Ribosome World may hold the secret [12] (Figure 1).

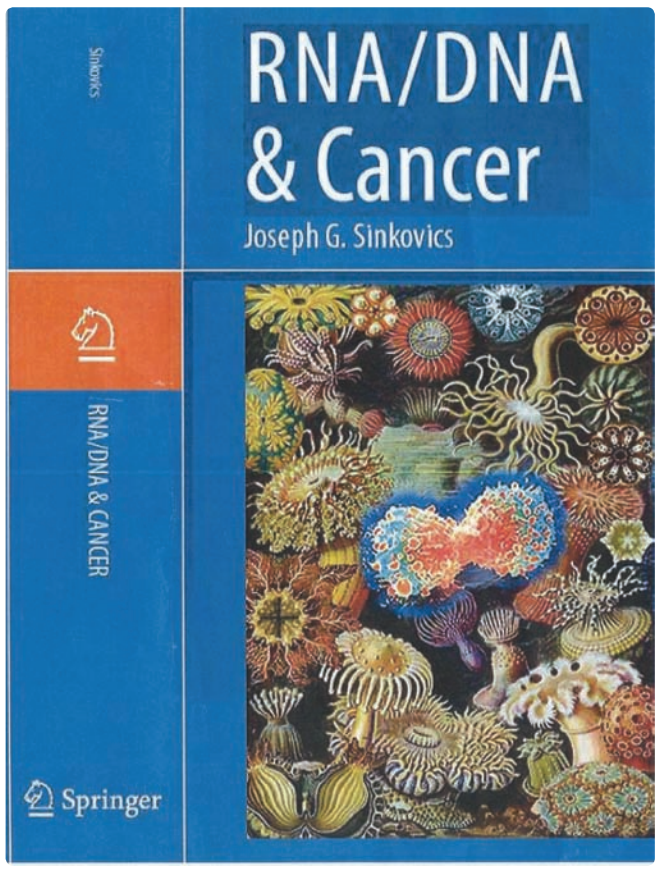

Figure 1: Cover page of the book RNA/DNA & CANCER Springer Verlag 2016.

Way before these events, Lynn Margulis, et al. [13] envisioned the first eukaryotes as fusion products of two primordial cells, for what she was repeatedly rejected and even reprimanded. Later, the two uniting cells were thought of being a prokaryota (bacterium) and an archaeota. This concept has been fully accepted, and further specified, as if the archaea was an Asgard Lokiarchaeota, and the mitochondrial donor an alphaproteobacterium, thereafter acting as a permanent endosymbiont in all cells of Animalia [14]. In all Plantae cells, the cytoplasmic endosymbionts became the pioneer cyanobacteria [15]. A scenario, in which the first cellular fusion partners were united by an ancient fusogenic virus, like that of the mycoplasma *Acholeplasma laidlawii*, is described in detail in the monograph RNA/DNA and Cancer [12,16].

THE PRINCIPLE OF RETROGRADE IMMORTALIZATION OF SELECTED EXTANT CELLS IN A MULTICELLULAR HOST

A semblance of, or actual immortality, is a prime characteristic feature of the natural unicellular life forms, primordial or extant [12]. Genomics and proteomics of the extant bilateral triploblastic, even vertebrate, descendants of the natural primordial unicellular and diploblastic multicellular life forms indicate the persistent existence of a conserved faculty, which when constitutively reactivated in selected single cells of these complex hosts, retrograde immortalizes these subjects. This preserved genetic feature within the evolving RNA/DNA complex is characterized by its high rate of somatic mutability. Cells in the process of transformation display an extraordinarily increased release of exosomes, which may have derived from ancient pre-cellular ribosomes. The enforced high rate of released exosomes is considered to be an atavistic recurrence: a regression to the ancient life forms consisting of pre-cellular ribosomes [17]. Pre-cellular ribosomes are thought to have been capable of originally uniting within themselves for the first time cooperatively the functioning metabolism, the compartmentalization, and the genomics together [12,17]. Ribozyme-armed pre-cellular ribosomes were probably capable of materializing the Virus World and Gene Pool envisioned by Koonin, Senkevich and Dolja, et al. [18].

The long latent survival of extant cells in the state of transformation and/or autophagy [19] develops into recoverability with new gene mutations occurring. These processes result in close to total resistance to physico-chemical insults. The signaling cascades for immortality are initiated by preserved retroviral genomic insertions. See braf/BRAF reactivation in a somatic mutation of malignant tumors (*vide infra*). In some of these processes, distantly separated genomic segments approximate one another in order to fuse permanently. Reactivated and *env*-ed retroviral particles may emerge to fuse cell membranes, thus favoring the natural generation of multicellular hybridomata. Such multinucleated large cells are characteristic of certain pathological entities (one example, the Reed-Sternberg cell). In human pathology, geneticists refer to fused oncogenes encoding oncoprotein. The clinics diagnose various forms of multicellular cancers [12].

The primordial ancestral eukaryotic life forms survived the physico-chemical adversarial turmoil into which they have evolved themselves in the planet Earth, by metabolizing like our current cancer cells do. In the genome of the predatory ctenophores, the ancient Wnt/ β-catenin pathway operates without the stop signals APC and Dickkopf, as it is in colon cancer cells of the human host (cited in [20]). Pre-Cambrian ancestors of the sea cucumber Echinoidea, utilized microtubule stabilizing proteins. The descendant microtubule stabilizing proteins EML1, 2 encoded by the human genome serve as stabilizer chaperons to on co-proteins ABL in acute T cell leukemia, or ALK in non-small cell lung cancer [21].

Independent immortal predatory single eukaryotic cells (amoeba-like), and diploblastic multicellular organisms (ctenophore-like), populated the ancient planet. Over time, selected descendants of the wild cells underwent some taming mutations, by the instalments of what is called the "tumor suppressor genes", the antagonists of the so-called proto-oncogenes. The tamed cells organized themselves into monumental societies. They retain in their individual RNA/DNA genomes the encoding of their ancient proto-oncogenic pathways for survival, if necessary in the state of autophagy, with full recovery, through new mutations [19]. The cell survival pathways of the genomes are capable to function alternatingly in the acquisition of their immune defenses, in developing resistance to chemical or radioactive confrontations, or in the ontogenesis and evolution of their progeny from diploblastic to triploblastic bilateral hosts. Ancient retroviral genomic elements permanently inserted within the conserved RNA/DNA complecti function as the faculty for the retrograde immortalization of some selected cells, primarily operational in stem cells, but not exempting fully differentiated somatic cells. This is documented below in one of its examples, as the

braf/BRAF oncogene/oncoprotein in papillary craniopharyngioma. The ancient cell survival pathways activated constitutively in some selected single cells of bilateral triploblastic hosts on the top of the evolutionary ladder (Homo) have become the proto-oncogenes/oncoprotein (*vide infra*). The process serves the sustenance of immortalized cellular life forms on Earth, and probably in the entire Universe [12,17,20,21]. Upon encounter with these cells in a triploblastic host, the clinics diagnose 'cancer'.

TWO DIFFERENT ENTITIES OF CRANIOPHARYNGIOMA

CP presents itself as an example of two different human malignant tumors: one, the adamantinomatous dysontogenic tumor deriving in young hosts from a faulty ontogenetic mechanism in the failed dissolution of a fetal organ; and the other, a papillary squamous cancer, afflicting hosts in their late adulthood, and emerging consequentially to newly acquired somatic genetic mutation in an ancient retroviral insert, the braf/BRAF (rat fibrosarcoma B). The first tumor is recognized as of 'benign histology, but malignant behavior'; when recurrent after surgical removal, it becomes malignant both in appearance and behavior. The second tumor displays full malignancy from its beginnings on, in all categories of its definition, with an eventually fatal outcome.

The adamantinomatous CP of children and young adults' forms a cystic suprasellar mass. The cysts contain a thick dark oily liquid containing cholesterol crystals. The cystic mass represents the fetal organ known as the Rathke's pouch (cleft, cyst, duct, fold), that was to be dissolved after birth. The normal involution of this fetal organ is carried out by an apoptotic process, which may fail. Thereafter, the disorganized growth of the cystic mass is encoded by the Wnt/β-catenin pathway (wingless, drosophila; integrated, mouse). This very same pathway in a switched-on/switched-off mechanism encodes the ontogenic development of the neural tube in vertebrate embryos. Several human cancers (colorectal, and many others) operate a mutated β-catenin (encoded from chromosome 3p21) gene [17,22]. Exon 3 of the β-catenin gene may be mutated so, that the protein molecule escapes phosphorylation, which otherwise would direct it to elimination by dissolution. In adamantinomatous CP, first frizzled and low-density lipoprotein cell surface receptors capture the ligand Wnt, and recruit the intracellular protein disheveled. Their complex fixes axin; the latter being a constituent of the cytoplasmic β-catenin destructive complex. In absence of a functional axin, the remaining members of the complex, APC and GSK (adenomatous polyposis coli; glycogen synthase kinase) fail to phosphorylate cytoplasmic β-catenin (even when it is not mutated), which thus escapes ubiquitination. Cytoplasmic β-catenin accumulates in excess. Excess cytoplasmic β-catenin eventually transgresses from cytoplasm into the nucleus, where it attaches to certain specific configurational sites in the genomic DNAs, representing a number of proto-oncogenes. The most prominent of these are c-myc/MYC, tcf/TCF, lef/LEF, and cyclin kinases (myelocytomatosis; T cell factor; lymphocyte enhancer factor; cell cycle initiator enzymes). These are genes of very ancient origin, which were possessed by the ancestors of diploblastic multicellular life forms (ctenophores; cnidaria; ciona) way before the appearance of late-comer lymphocytes of bilateral triploblastic hosts, which acquired the ancient genes by evolutionary inheritance. These genes assumed the functions of proto-oncogenes in hosts higher up in the evolutionary scale in all of the multicellular descendants (including Homo). These amplified and later potentially constitutively activated genes have become the drivers of so-called malignant cell proliferations, clinically diagnosed as adenocarcinomata. When the β-catenin gene is not mutated (as in some adamantinomatous CP; others carry mutated exon 3 in the ctnnb1/CTNNB1 gene/protein). Its expression may be regulated by silencing methylations in the promoters of the genes operational in the β-catenin destructive complex. Tumors induced by not-mutated β-catenin may thus remain in the grade 1 histopathological category. In adamantinomatous CP, one of the further genomic alterations is deficient or absent, or excessive: galectins, inducers of the physiological apoptotic cascade, which is missing, or non-functional. Excessive expressions of 'wet keratin', fascins, nestin, EGF-R, PDGF-R, FGF, and VEGF are present (epidermal growth factor GF receptor; platelet-derived GF receptor; fibroblast GF; vascular endothelial GF) [22,23].

These tumors are removed by neurosurgical procedures. Incompletely removed tumors are treated with radiotherapy and investigational bio-chemotherapy including tamoxifen, temozolomide, interferon (IFNα), the Streptomyces verticillus antibiotic bleomycin, other chemotherapeutic agents, etc, since one particular standard proven-effective treatment for the recurrent tumors does not appear to exist, or has not been generally accepted. When interferon-α is used, it is not clear if it was to treat an additional JAK/STAT mutation (Janus-kinase; signal transducer activator of transcription), or if these signals were IFNα-induced. This tumor fulfils all classical concepts of the faculty 'ontogenic carcinogenesis' of the textbooks. An example of this is the hepatoblastoma family [24]. Adamantinomatous CP is not originating from mutated stem cells. A faulty system within ontogenesis, kept alive by a consequential driver pathway (Wnt/β-catenin) automatically sustained. Additional mutations (a sonic Hedgehog pathway) may emerge [25], creating an immortalized cell population with persistence and local growth, escaping immunological rejection, but seldom if ever producing distant metastases. Vast numbers of literature appeared [26,27], as if the β-catenin molecule may be non-mutated wild-type, or mutated at its GSK-binding site [28]; or as if malignantly transformed stem cells could appear in this tumor [29]. Non-mutated β-catenin remains in the cytoplasm; mutated β-catenin accumulates within the nucleus, a critical event for increased cell replication [30ab].

An elderly patient develops bitemporal hemianopsia due to a suprasellar mass above the chiasm. By biopsy the tissue diagnosis of the tumor is that of a 'papillary craniopharyngioma'. The tumor cells carry the single nucleotide somatic mutation V600E of the braf/Braf gene/oncoprotein (Werner Kirsten's rat fibrosarcoma B), in which instead of valine, glutamate (glutamic acid) is encoded in the protein product of the gene [30ab]. This is the driving mutation recognized in several other human tumors, in which the tumor cells are not trans-speciated, but retain their original physiological morphology (malignant melanoma, thyroid carcinoma, hairy cell leukemia, etc). In this case, the CP cells become those of a keratinizing squamous carcinoma. These cells undergo immortalization, dividing frequently, and metastasizing as highly chemo-radiotherapy-resistant individuals. They extricate themselves from the tightly organized cell community of their hosts. Further, these tumor cells may initiate several close to innumerable further metabolic somatic mutations, not involving the so-called proto-oncogenes. Actually, new second oncogenic driver mutations may also occur, but in braf-gene-mutated papillary CP

cells the β-catenin gene is never co-mutated [30ab]; however the Ki67 values are high.

Tumor cells driven by mutated, fused, and/or constitutively activated proto-oncogenes-oncoproteins will propagate within their multicellular community as entirely independent individuals entitled to full immunological protection, even stimulation, consisting of the receipt of full support with nutrients and growth factors through increased vascularization. In these tumors the so-called tumor suppressor genes had already been eliminated. Eventually, these tumor cells will overgrow and kill their host, unless highly specific small molecular inhibitors are found and applied to fit precisely into the genomic configurations of the involved mutated nucleotides, thus inhibiting their reproduction. Vemurafenib, or dabrafenib are these inhibitors in the case the V600Ebraf/Braf mutations. In addition to Braf mutations, the catenin gene/protein ctnnb1/CTNNB1 may be mutated, and/or the MAPK/ERK (MEK) pathway (mitogen-activated protein and extracellular signal-regulated kinase kinase) may be constitutively activated [26,27,31]. The MEK mutated genes may be targeted by trametinib, and their protein-products may be neutralized by monoclonal antibodies (to be discovered). Here, an anciently inserted retroviral genomic fragment induced retrogradely the immortalization of a few selected cells resulting in 'malignant behavior' in their multicellular host [30ab,31].

OTHER TUMORS OF CONCERN

Should the original tumor be an adenocarcinoma, and in it secondarily the Achaete Scute (AS) proto-oncogene be mutated (chaete, drosophila bristle), and thus constitutively activated, the newly emerging tumor cells become those of a malignant neuroectodermal tumor. In this case, the inducer human proto-oncogene/oncoprotein ash/ASH is the responsible descendant of its cnidarian or amphioxus (*Branchiostoma floridae*) ancestors. There, the original achaete-scute gene created the very first nerve cells. As a primary driver, an achaete scute protooncogene induces the human malignant tumor, esthesioneuroblastoma of the nasal olfactory ganglia (see full story in the RNA/DNA and Cancer [12]).

In the case of malignant melanoma, monoclonal antibodies (mcab) neutralize the ligands and/or their receptors in the T lymphocyte-embedded immune checkpoint networks (Allison, et al. [32ab]). These mcab are specific to receptors CTLA-4, or to PD1 of auto-reactive T lymphocytes, and to the ligand PD1L1 thereof (cluster of differentiation CD152; cytotoxic T lymphocyte-associated; programmed death and its ligands). The ligands cannot activate their antibody-coated receptors, thus the receptors refrain from the induction of apoptotic death of their own autoreactive T cells. Instead, the auto-reactive T cells attack, especially the tumor cells, which over-simulate their selfness and overproduce the ligands in vain. Such potentially tumor-curative monoclonal antibodies are ipilimumab, nivolumab, pembrolizumab, atezolizumab, etc. As collateral damage, some healthy parenchymal structures have to withstand a moderate corticosteroid-sensitive autoimmune attack. As an exceptional case, a middle aged male patient of this author was sent to the National Cancer Institute for the treatment of disseminated melanoma with ipilimumab. He died of corticosteroids-resistant autoimmune hemorrhagic enterocolitis. Kaplan-Meier graphs show current long term tumor-free survival rates, and/or progression-free long survivals of patients with metastatic melanoma treated with these single or doubled monoclonal antibodies from the Massachussetts Medical Society (Robert, et al. [33]). However, some transformed cells are able to recover from these immune blockades, and the patients still relapse (Robert, et al. [33]). For example, protracted interferon production or administration is antagonistic to the efficiency of checkpoint blockade [34]. Combined use of two different monoclonal antibodies (or even in combination with targeted therapy, or chemotherapy) predicts better results (actual cures?). The chimeric antigen-modified CAR immune T cells might have already approached that result, at the risk of inadvertently induced cytokine storms with brain edema (referring to recent large literature cited elsewhere). That is, the host with external help will be able to overcome the natural process of retrograde cellular immortalization, even though the genomics of that has been inherently inscribed in its primordial RNA/DNA complex.

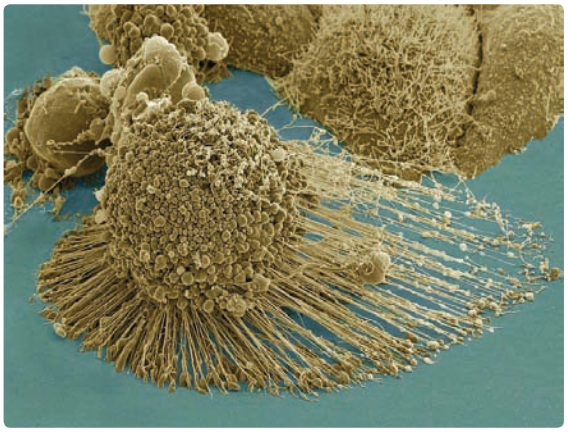

Figure 2: Scanning EM micrograph (Zeiss Merlin HR-SEM) of an apoptotic HeLa cell from the Rocky Mountains Laboratories, Hamilton, Montana, as shown in Google and Wikipedia. This HeLa cell lost its immortality, but retained its susceptibility to oncolytic Newcastle disease virus (NDV) (Sun et al. [64]).

INDEPENDENTLY FREE LIVING IMMORTALIZED (CANCER) CELLS

If immortalized cells (the cancer cells) are transferred into an environment providing for their full nutritional needs (a tissue culture in an academic laboratory), these cells may preserve their full chemo-radiation resistance, or eventually lose it. Some of the original HeLa cells after decades in culture, move like large amoebae, invade cultures of other cancer cells (accidentally, admixtures occurring in laboratories maintaining several different cancer cell lines), fuse with the other cells, phagocytose other cells, unite their own genes with those of the engulfed counterpart cell, in newly generated fusion products; or, become susceptible to apoptotic death, thus losing their immortality (Figure 2: EM photograph of an apoptotic HeLa cell from the Rocky Mountains Laboratory, as shown in Google and Wikipedia). HeLa cells losing their immortality could have united in a colony. HeLa cells' mRNAs responding to oncolytic Newcastle disease virus (NDV) (Sun, et al. [64]). Nourished well, and exempted of either the support, or the attacks of a multicellular host, cultured cancer cells become entirely self-sufficient, and independent (the HeLa cells in pp. 302 and 479; or human melanoma sarcoma and carcinoma cells cultured long term in the 1970s at the M.D. Anderson Hospital, pp. 337 and 479-480 [12]). They remain subjects to any other future mutational changes in the repertoire of a much multifaceted RNA/DNA genome. Reactivated fusogenic retroviruses

remain their original allies [12]. Settled in a distant metabolically favorable environment, there exempted of any hostile reception by a pre-existing population of established cell communities, these cells could sustain their life style even under adverse physico-chemical conditions. After further somewhat taming mutations, they possibly could form gradually organizing communities and evolve further into large cellular societies.

Would those scientists, prominently the astrobiologists, who consider the arrival of such cells via meteorites to the ancient Earth, believe that they might have derived from 'malignantly transformed' predecessors from distant planets, and that the surviving cells in the hostile environment of the ancient Earth metabolized as the extant 'malignantly transformed cells' do; and eventually organized themselves into the present taxa and species of organized cell communities (certainly not entirely tamed)? Or, that the meteorite-bombarded ancient Earth has already released such ever-resistant ancient cells and their RNA/DNA complecti into our Universe? The ancient RNA/DNA complex possessed and latently preserved its aboriginal faculty to retrogradely immortalize extant cells for a haphazard dispersal on Earth, or in the Universe, for the preservation of cellular life, as originally constructed, anywhere in the Universe/ Multiverse). The first eukaryotes populating the ancient Earth might have had to metabolize for survival, as some selected extant descendants of them, the cancer cells, do. A newly generated such cell population would not be allowed to expand on Earth now, due to the Earth's widely established biological flora. Even the mammalian vertebrate host's resident lymphocyte flora has to be solidly decimated in advance in order to allow the establishment of newly infused allogeneic, or even autologous, lymphocyte populations (clinically utilized for therapeutic purposes). However, tumor cells are known to spread cancers in nature, specifically among the wild population of the Tashmanian devils (Sarcophilus harrisii), and in soft shell bivalve clams (Mya arenaria) in the oceans (cited in [12]). In the case of the bivalve clams, the tetraploid tumor cells survive in sea water, as they move from afflicted clams to healthy ones. Even though the tumor cells are endowed by the driver retrotransposon Steamer, they are not known to replicate outside their host organisms [35]. Is it possible to maintain them in long term cell cultures? Could retrogradely immortalized cells colonize their inanimate environment? The first eukaryotes populating the ancient Earth might have had to metabolize for survival in an alien environment, as extant cancer cells do in permanent cultures.

It was reported first in the Lancet in January 1970, that retrovirus-producer diploid mouse lymphoma cells fused with antiviral antibody-secreting immune mouse plasma cells of the same strain, thus creating natural tetraploid hybridomas (this terminology was not used in that article). The tetraploid tumor cells grew invasively and in the form of large hemorrhagic ascitic tumors in the mouse, and could be maintained in spinner bottle suspension cultures for over ten years (in Trujillo's lab), while producing the specific leukemia virus neutralizing immunoglobulin antibody, and releasing deformed non-infectious retrovirus particles [36-41]. By imaginary analogy, the possibility that the large multinucleated Reed-Sternberg cells are similar natural hybridomata deserves further studies [12,41].

DISCUSSION

The ancient cell communities evolved genomes encoding pathways for the ontogenesis of their progenies, and/or acting in self-defense against physicochemical and biological intruders. Single celled eukaryotic life forms (unikonta, exemplified by amoebozoa; including the multicellular colonies of the highly talented Dictyostelium discoideum, and the syncytial Physarium polycephalum [12]) must had been pre-occupied with their individual survival and replication, while preventing senescence and death. Their metabolic pathways travelled through evolutionary routes from multicellular diploblastic to bilateral triploblastic hosts (including Homo). Extant derivatives of the original sea cucumbers micro-tubule proteins are the protector chaperons of some human oncoproteins [21]. For widely shared functions, such as ontogenesis, immune defense, and oncogenesis (here referred to as retrograde cellular immortalization), common shared pathways remained active as ranks 1-11, to follow. In rank 1 are the NFκB/STAT (nuclear factor kappa B lymphoma; signal transfer activator of transduction) pathways from the diploblastic cnidarians (*Nematostella vectensis*) to reappearing in the human genome as oncogenes in mediastinal lymphomas and in Reed-Sternberg cells of Hodgkin's lymphoma [20]. Both the ancient and the current processes are associated with viral particles of questionable activity [20]. Pathways, as 2, are PI3Ks (phosphatidyl inositol kinase) in the Giardia; 3, the WNT/β-catenin, incomplete in ctenophores, full in *cnidarians* [20], and *ciona* [17]; 4, the TGF-β (transforming growth factor) in placozoan ctenophores; 5, the hedgehogs, hedge appearing before hog from choanoflagellata to sponges; 6, the nuclear receptors, already present in choanoflagellata; 7, the notch-δ, operational in choanoflagellata Monosiga brevicollis, Capsaspora owczarzaki, placozoan ctenophores, cnidarians, and sponges; 8, the protein tyrosine kinases, receptor tyrosine kinases widely spread (PTK, RTK); 9, the ras/RAS/MAPK (rat sarcoma; mitogen-activated protein kinase), originally named to be Class I oncogenes carried in retroviruses (Spandidos [42]); and 10, the Growth Factor (GF) precursors (fibroblast FGF, vascular endothelial VEGF) appearing already in choanoflagellates, ctenophores, cnidarians (*Nematostella vectensis*; *Hydra viridis/ viridissima*) and sponges, all travelling from the state of ancient ancestors to their extant descendants [12]. The rank 11, JAK / STAT pathway united itself in bilateral advanced life forms, due to the late appearance of JAK; JAK has been a bilateral innovation (Babonis & Martindale [43]). All descendants of all of these ancient survival pathways genuinely practice retrograde immortalization in the human genome [12]. Added at the end is the most recently elaborated-on pathway: the rank 12, VDAC/ HxK (vide infra).

Further, in the first multicellular life forms (in the gonidia of the *volvocales, Volvox*, cited in [12]), undifferentiated stem cells are awaiting to receive the message to generate a replacement somatic cell, while preserving their genomes for self-reproduction as well; fully differentiated somatic cells are engaged in a one and only particular function (swimming to light); and germ cells are destined to be zygotes. These cells in all other similar systems arrive at the stage of full constitutive retroactive de-differentiation (example, Hydractinia, cited in [12]) through many possible avenues.

External physicochemical intruders (gamma ϒ/X-ray/UV irradiations) induce DNA repair enzymes, that may secure survival of the cell even in the case of a genomic catastrophe (the Namalva Burkitt's endopolyploid lymphoma cells recovering from 10 Gy X-ray irradiation, cited on pp 197-8 [12]). ATP-binding cassette ABC proteins

rise to expel indigestible toxins (Table I in [12]). All these cells arrive at the stage of full constitutive retroactive de-differentiation through many possible avenues. Transformed (retrogradely immortalized) cells survive chemo-irradiation in the state of autophagy, and recover after the initiation of other new mutations, or suffer Dobzhansky's synthetic lethality (Table X in [12]). When distant gene pairs are undergoing point mutations synchronously, the cells, harboring these double mutations, die. Recognizing one of these two genes allows the identification of its determinate pair by a combinatorial CRISP-Cas9 (clustered regularly interspaced short palindromic repeats cascade) screen utilizing G-C versus A-U interactions of primary hairpin loop gRNA (guide) scaffolds. Targeting that second gene directly to mutate, induces synthetic lethality of that cell, already malignant, or eventually going to be [44]. When PI3K-induces the proto-oncogene mTOR (mammalian target of rapamycin) in giant astrocytoma cells of tuberous sclerosis, everolimus is its specific inhibitor [45]. Another genome-specific inhibitor of the V600E mutation is dabrafenib. However, MEK-driven tumor cells emerge. The MEK-specific inhibitor is trametinib, that also reduces the incidence of the cutaneous hyperkeratotic squamous cell carcinoma potentially induced by vemurafenib [27,30ab,46]. Thus, the cell cycle can be halted before its irreversible roll.

Inhibitors of autophagy, or autophagosome formation, by 3 CH_3-adenin, chloroquine and others, combined with chemo-irradiation, prevent chemotherapy-resistant mutations in the cells trying to enter autophagy. These cells are directed to surrender irreversibly to their death. Thus, such a combined attack can kill the pre-autophagic cell before its recovery with new oncogenic mutations (that chemotherapy may rather promote, than inhibit). The tumor suppressor genes act as natural inhibitors of autophagy (however, transformed cells usually already deleted or suppressed these genes) [47-49]. However, if retained in an inactivated state, but not necessarily in autophagy, the malignantly transformed cells (human promyelocytic leukemia, or chondrosarcoma cells) may be able to re-differentiate [17,50], meaning, suppressing their faculty of retrograde immortalization.

The evolutionary appearance of placental mammalians fundamentally altered the evolving adaptive immune system, as it allowed the standard acceptance, and induced the immune biological support for, alien fetal tissues by the maternal immune system (specifically, for the allogeneic paternal antigens in the fetal cells). It is the placental IDO1 system and its derivatives that make this possible. Host cells in the process of endogenously initiated transformation, i.e. the inherent retrograde immortalization, imitate the placental trophoblast by expressing its major immunosuppressive proteins, the IDOs (indole-amine 2,3-dioxygenase) [51,52]. Biological intruders are confronted by a complex, highly evolved and combined native and adaptive immune system operational with Treg stop signals (reviewed in pp. 105-125 [12]). However, the endogenous internal induction of cellular dedifferentiation, i.e. retrograde immortalization, imitate the placental trophoblasts by anciently inserted and preserved retroviral genomic segments (syncytins) and IDOs, leading to the ultimate constitutive and irreversible activation of some primordial survival pathways of the invading cells.

Genomic events are often initiated and sustained chronically in inflammasomes [53,54]. These are the driving mutations of those dedifferentiated cells, which are privileged to receive full support by the faculties of their afflicted hosts. All defensive immune reactions are deflected and replaced by full support with growth factors and neovascularization directed at the targeted cells. Cells in this natural stage of transformation, will mobilize all ancient survival pathways held in resort, in response to exposure to chemo-radiotherapy, as their ancestors did in the occasions of numerous affronts in the micro environments of the ancient Earth, with incompatible external physico-chemical agents. Even in the stages of autophagy, these cells will be able to mobilize new mutations for full recovery and survival. If the tumor-bearing patient received immunization with a vaccine (a viral oncolysate [55]) directed against the oncoprotein generated in the original first mutation, the same vaccine will be ineffective against the oncoproteins of the second or third mutations in the relapsed tumor. Thus, originally generated immune T cells become ineffective, but NK cells are expected to remain potentially effective, irrespective of the numbers of sequential mutations in the targeted cells [12,55]. Chronic inflammations favor the induction of retrograde immortalization of some selected single cells of the host [53,54].

A way to overcome the transformed cell is by releasing the forbidden anti-self-reactive immune faculties of the host. By inhibiting the essential contacts of the ligands with their appropriate receptors that induce apoptotic death of the anti-self-reactor immune T lymphocytes, the lymphocytes now liberated, deliver their lethal auto-immune attacks on the tumor cells, which were masquerading as if they were genuine self-elements exempted from such attacks [32ab], but rather entitled to receive support. The process of disguising (simulating) selfness, renders the tumor cells to be genuine targets for the liberated autoimmune T lymphocytes, at the cost of unwanted genuine autoimmune reactions in normal tissues (thyroiditis; hypophysitis; ophthalmitis; severe hemorrhagic colitis) exerted by the liberated clones of autoimmune T lymphocytes. However, even that anti-tumor attack may fail, as the transformed cells have recently shown that they may be able to mobilize elements for recovery from genuine autoimmune lymphocyte-mediated attacks, when the autoimmune lymphocytes were preserved and rescued by mcab like nivolumab or pembrolizumab [56].

The biological importance of transformed cells (malignantly transformed cells; tumor cells; retrogradely immortalized cells) is clearly recognized by the multicellular diplo- or triploblastic hosts, inasmuch as inbuilt natural immune checkpoint systems are operational in the latter hosts for the protection and support of these cellular products [57,58], as they present themselves in the disguise of genuine self-elements. Grossly, these protective systems appear suicidal, in as much as the protected transformed cells eventually kill their hosts and die within them. However, in refinement of their scope, certain individual transformed cells liberated after the death of their original host, could remain alive and as such, may be able to replicate in a new environment appropriate to their metabolism. These cells could sustain RNA/DNA-encoded cellular life in the Universe, under adverse physico-chemical circumstances, what the original primordial faculty of generating biologically valuable retrogradely immortalized cells is determinant to serve.

UPDATING OF THIS SUBJECT MATTER

The billion years' old ancient relationship of proteo-bacteria with the first eukaryotes reveals a key element of their profound biological association. The pores of the proteobacterial mitochondrial

membranes' Voltage-Dependent Anion Channel (VDAC) render these structures permeable for the flux of ions and metabolites (prominently adenosine triphosphate, ATP) into and from between the host cytosol and mitochondrial plasma. VDACs 1, 2, 3 exert distinct functions; VDAC1 is pro-, VDAC2 is anti-apoptotic. Crucially, the glycolytic hexokinases (HxKI, II, and III) and Bcl-2 family proteins protect the cellular nuclear DNA strands from apoptotic disintegration in cells undergoing malignant transformation (or retrograde immortalization, by the current vocabulary). The first sugar-metabolizing hexokinases appeared in the archaea, *Sulfolobus, Thermoproteus* and *Pyrococcus*, and in all prokaryota. Eukaryota might have acquired them from the mitochondria (proteobacteria) in Animalia, including all unicellular protozoa (*Cryptosporidium, Entamoeba, Leishmania, Trypanosoma*); and in all Plantae from chloroplasts of cyanobacterial origin. Yeasts (*Saccharomyces*) and all fungi (*Aspergillus*, *Cryptococcus*, *Magnaporthe*, *Yarrowia*, the ascomycete *Neurospora*), mosses (*Physcomitrella*) and all plants followed suit. It is in the bilateral triploblastic multicellular macro-organisms (including Homo), where diversification of hexokinases occurred from essential sugar metabolizing molecules toward anti-apoptotic proto-oncogenic proteins. Hypoxia-Inducible Factor (HIF) up regulates HxK I, II production and induces C-terminus-defective VDAC1ΔC. Ras-transformed fibroblasts with inactivated Vdac −/−/ VDAC−/− in their network of intra cytoplasmic fused mitochondria, entered the state of hypoxia due to activated HIF-1. These cells with activated Extracellular Signal-Regulated Kinase (ERK) assumed accelerated replication due to reduced expression of tumor suppressor gene/protein cdkn2α/CDK (Cyclin-Dependent Kinase) [59,60]. Mitochondrial VDAC1 genes are transferred to the cell nucleus; from there, the proteins VDAC1 are synthesized via mRNAs in cytoplasmic ribosomes, and VDAC1 proteins from there are transferred into the mitochondrial membranes. This pathway terminates in mitochondrially induced cell death. HxKII proteins inhibit such translocations by producing HxKII and VDAC1 complexes; thus, malignantly transformed cells over produce HxKII proteins in order to rescue tumors from apoptotic death. HxKII proteins by their first 10 to 20 amino acids bind VDAC1 in the mitochondrial membrane. Five AA from histidine to proline are essential for the binding. The HxKII/ VDAC1 bond in the mitochondrial membrane is a major blockade to apoptosis induction, as the pro-apoptotic VDAC1 is neutralized by HxKII. HxK proteins still phosphorylate glucose molecules in the pentose phosphate pathway, as they have done in the first cells on Earth, thus supporting cell metabolism and replication. HxK synthesis can be measured by Western blotting and blocked by the iRNA shHxK pathways [61,62]. The vertebrate mammalian HxKII genes are recognized as anti-apoptotic proto-oncogenes, and are to be targeted as such. Aspirin is an apoptosis inducer in many different cancer (HeLa; Jurkat) cells by augmenting ATP and ionomycin-induced mitochondrial Ca^{++} uptake, up-regulating pro-apoptotic bax/ Bax, downregulating anti-apoptotic bclxL/BCLxL, and separating away the VDAC membrane-bond HxKII. It fails to induce apoptosis in cells with siRNA-silenced VDACI mitochondrial membranes [63]. In addition to the numerous established pathways serving the cell in its ontogenesis, metabolism, survival in distress, immunological defense, evolution, and retrograde immortalization (malignant transformation), as listed above in ranks 1-12 [12,43], the VDAC mitochondrial membranes retained from the deep past and united with unique derivatives of the ancient cellular proteins HxK are essential components of the cells' life or death machinery.

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