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CONTRIBUTIONS OF EVOLUTIONARY BIOLOGY TO THE UNDERSTANDING OF CANCER

IONESCU-TIRGOVISTE CONSTANTIN[1], MATEI IOAN VALENTIN[2], IVANUS BOGDAN[2] and FALCAN ANA MARIA[3]

[1]National Institute of Diabetes, Nutrition and Metabolic Diseases "N.C. Paulescu" Bucharest
[2]Clinical Emergency Hospital "Sf Ioan" Bucharest
[3]University of Medicine and Pharmacy "Carol Davila" Bucharest
Corresponding author: Constantin IONESCU-TIRGOVISTE, E-mail: cit@paulescu.ro

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Evolutionary biology has proven itself to be a useful tool for medical oncologists and cancer researchers. There is a growing body of evidence that suggests the emergence of cancer is itself a Darwinian process, which abides by the rules of natural selection. Non-Darwinian evolutionary processes such as genetic drift also play an important part. The consequences of these findings are pivotal to the understanding of the failure that attempts at treatment have encountered thus far, as well as to the design of future therapeutic approaches.

Key words: cancer, carcinogenesis, evolutionary theory, clonal evolution, acquired drug resistance.

INTRODUCTION

While evolutionary biology is, by and large, concerned with the study of individual organisms and populations, molecular biologists have long since regarded cancer development as an evolutionary process, in which natural selection favors certain cells that are able to survive in hostile environments, develop new metabolic circuitry, as well as pathways of aggression and resistance[1,2]. Cancer was first described as an evolutionary system by Nowell in his 1976 article, postulating that "acquired genetic variability within the original clone allow(s) sequential selection of more aggressive sublines"[3]. Evolutionary forces function on many levels in biology – among somatic cells within an organism, as well as among organisms themselves[4]. Ecological interact-tions such as predation and competition can be successfully applied to the somatic evolution of cancer as well[1].

In order to gain perspective on cancer at cellular, tisular as well as organism levels, an evolutionary perspective is required. For multicellular organisms to appear, the development of strong antitumor regulatory mechanisms was in order, regulatory mechanisms that operate at the level of each cell, as well as that of tissues and body. The development of cancer entails the acquisition of mutations which give cancer cells an advantage over normal cells, allowing them to grow and spread from an insignificant initial tumor mass all the way to disseminated metastatic disease[5]. Cancer risk has been seen as a consequence of the combination between multicellularity, cell turn-over, as well as genetic and epigenetic changes occurring over prolonged spans of time. With cells in the same organisms being, for the most part, genetically identical, the origin of each genetically distinct cancer cell line can be compared with the sympatric origin of new asexual species that enter into competition with neighboring as well as progenitor cells for resources and space[1].

Indeed, recent theories view carcinogenesis as a form of speciation. According to Duesberg *et al.*,

carcinogenic agents cause aneuploidy, which destabilizes the karyotype, leading to the emergence of ever-more random karyotypes automatically. The minority of these that achieve reproductive autonomy can be said to have gained the primary characteristic of both cancer cells and species. The speciation theory presents a number of advantages, in that it elegantly accounts for several of the hallmarks of cancer[6], as well as the phylogenetic relations between cancers in the same tissue, and solves the paradoxes of other cancer theories, more specifically, "cancer's fatal flaw", according to which aneuploidy would be expected to impair normal growth and development; if the aneuploidies in neoplasms are karyotypes of new species, the (apparent) paradox is solved[7]. Moreover, this accounts for the behavior of metastasis, which can be regarded as an aggressive invasive species that migrates to new environments and takes over the niche occupied by the original inhabitants, either by killing them or successfully competing for the available resources. Migratory ability, while maladaptive in homogenous tumors, gives malignancies an adaptive edge, allowing cancer clones to prospect for more suitable environments within the organism[5].

From an evolutionary perspective, cancers are large and genetically diverse populations of individual cells. Genetic and epigenetic modifications beneficial to a certain cellular clone, aiding its expansion, are ultimately deleterious to the host organism, leading to its death and, consequently, also that of the neoplasm. Mutant clones within a neoplasm expand or contract by natural selection and genetic drift, despite any harmful effects they might have on the host body. Each cell's Darwinian fitness is determined by its relationship with neighboring cells and a wide array of factors in its surroundings, including therapeutic intervention of any kind. Selection favors increased proliferation and survival, the consequences of which are invasion, metastasis and resistance to treatment[4].

Tracking clones as they evolve over time has proven to be an excellent means of studying evolution in neoplasms. Such studies have been reported for a series of conditions, including esophageal squamous-cell carcinoma, Barrett's esophagus, oral leukoplakia and ulcerative colitis.[4] Barrett's esophagus has been shown to be an ideal model for studying clonal evolution in cancer, enabling the spatial and temporal assessment of each genetic stage in neoplastic progression[1,8]. Aptly regarding neoplastic progression as a process of natural selection, the rate of evolution depends on mutation rate, population size and the intensity of selection. All necessary components of natural selection in a neoplasm have been confirmed for Barrett's esophagus – somatic variation, its heritability, as well as differing Darwinian fitness of clones arising from the variation[8].

MUTATION

Heritable variation within the population is an essential prerequisite for evolution, with more variation resulting in a faster rate of evolution[1,4]. Many cancers are characterized by a degree of genomic instability, which leads to an increase in genetic variation among cells, crucially important for the development of malignancy[1]. For Barrett's esophagus it has been proven that each separate clone accounts for an increased risk of esophageal cancer by a factor of 1.4, and that for every 10% of genetic divergence between cell lines, the risk factor would be further increased to 1.6[1,8]. According to recent studies, apoptosis and genome stability do not share an evolutionary origin, despite the web of interactions established between them. The network responsible for genome stability has most likely emerged earlier throughout the evolution of eukaryotic cells. Around 76% of the genome stability components emerged early in the evolution of eukaryotic cells. At the same time 39% of the apoptotic machinery, including some of the earliest apoptotic core genes (such as that for cytochrome c) emerged. Closer to the metazoan origin, key components are added to the intrinsic apoptotic pathway (such as BCL2 and the caspase family). Lastly, apoptosis is enriched with the addition of components to the extrinsic apoptotic pathway (such as the TNF superfamily). It was also found that older, less plastic and more essential genes were involved in genome stability. Apoptosis, on the other hand, is controlled by genes that are, by and large, more recent, more plastic and less essential. Genes responsible for genome stability are rooted as early as possible in the course of natural history[9]. Loss of genetic stability being a key phenomenon in carcinogenesis, researchers have proved that genetic instability arises due to the disadvantage

conferred by DNA repair in mutagenic environments. Thus, mutagenic environments not only produce mutations directly, but also select for cells that do not invest time and resources in repairing them, gaining an advantage over those who do[10].

p53 has been dubbed "the guardian of the genome" because of the crucial role it plays in cell cycle regulation. Mutations in the p53 gene occur in at least half of all human cancers[11,12]. Although the loss of p53 occurs relatively late in the course of carcinogenesis, impaired cell cycle-arrest and p53-mediated apoptosis in response to DNA damage gives the neoplastic cells an advantage relative to the wildtype, in enabling them to survive and divide while incurring significant damage to their genetic material[4,10].

Epigenetic alterations also play a key role in cancer development, with changes in the pattern of methylation accounting for a higher rate of neoplastic transformation than mutation does. Genes that are pivotal in DNA-damage response and repair, such as MLH1, MLH3. MSH6 and SFN are inactivated by hypermethylation, leading to a high level of genomic instability[4,13–16].

NATURAL SELECTION

As in the case of organisms, natural selection in cancer cells occurs through bitter competition for space and resources[17]. Cells with proliferating potential situated within the same tissue compete for nutrients, growth factors, survival factors and space. Collectively, all of these factors constitute an ecological niche[5]. Limited resources, as well as constraints posed by their respective micro-environments limit the size of tumor masses. There is a difference between the growth rate of cancer cells and that of tumors themselves, which is significantly larger for cells, suggesting that most cells die off before they are able to divide[17], on account of the significant selective pressure they are subjected to. Among the micro-environmental forces that induce genetic instability and account for the struggle for survival and reproduction, the ones considered most potent are hypoxia, acidosis and the presence of reactive oxygen species. Chemotherapy agents might also be added, on account of the increase in evolutionary rate they account for[18].

Generally, it is considered that, much like adapted organisms themselves, highly fit stem cell populations should naturally oppose somatic cell evolution, based on the rationale that a new mutation is much more likely to be maladaptive than to provide a further advantage. Competition between vigorous stem cells serves a homeostatic purpose, suppressing abnormal variants through competition with their fitter, better adapted neighbors. Cancers are, therefore, more likely to appear when general cellular fitness is lowered as a consequence of exposure to carcinogens or with aging, when oncogenic mutations gain an adaptive edge. They are adaptive in the sense of repairing the original damage or finding ways to work around it and, as is the case with all adaptations, can become maladaptive under changing circumstances. For instance, exposure to agents that destroy large swathes of cell populations favors mutations that promote cell survival. The effect is amplified by alterations to the ecological niche itself, in the same way antibiotic treatment selects for resistant germs, and generates further selective pressure through the destruction of commensal flora, compromising the local ecosystem[5]. When an adaptive mutation increases the Darwinian fitness of a clone, this often leads to what is known as a "selective sweep", in which this mutation increases its frequency in the population, eventually becoming fixed[4].

Onset of cancer entails a series of mutations at key points in the genome, resulting in the acquisition of what are known as the "six hallmarks of cancer", elaborated by Hanahan and Weinberg[6]. These comprise of a series of biologic capabilities that constitute the organizing principle for rationalizing the complexities of neoplastic disease: sustaining proliferative signaling, loss of sensitivity to growth suppressors, resistance to apoptosis, replicative immortality through telomerase expression, sustained angiogenesis, as well as invasion and metastasis[1,6]. Two additional emerging hallmarks can be added to this list – reprogramming the energy metabolism and bypassing immune mechanisms of defense[6].

All of the hallmarks lead to differential success of the respective clone.[4] This is especially true of tissues subject to recurring injury, where successive cycles of apoptosis and proliferation enable mutant clones with reproductive advantages to succeed[4]. The hallmarks of cancer can be aptly

described as the results of successfully overcoming selection barriers by the neoplastic process[5].

When drugs and radiotherapy are administered by physicians, the cancer clone dynamics are altered, but, nonetheless, any further developments will still follow an evolutionary pattern. This form of "artificial" selection destroys many cells while creating selective pressure for the emergence of new variants that are able to withstand the noxious stimuli. Moreover, since cancer treatments often manifest specific toxicity for genetic material, the new variants suffer mutations which may increase their reproductive success[17]. Cancer therapies have been shown to cause positive selection either through genetic variation in cancer stem cells, or through non-genetic means, such as signaling plasticity, quiescence and epigenetic change.[19] We will further discuss the phenomenon in the context of acquired drug resistance and the failure of cancer therapies.

GENETIC DRIFT

Other researchers view carcinogenesis through the lens of non-darwinian evolutionary models, as a fundamentally non-adaptive process[20]. According to Aranda-Anzaldo, it is genetic drift rather than natural selection that is the main driving force behind tumor growth and progression. In his view, cancer results from a diminishing of selective pressures – be it certain principles or developmental constraints – that ensure genome stability. It therefore follows that cancer has epigenetic rather than genetic causes, as it is determined by processes situated downstream from the level of genetic information[20].

The role of genetic drift can be ascertained by assessing variables such as effective population size, cell generation time and cell turnover, the latter of which, in turn, depends on the rates of cell division and programmed cell death.[4] When the population is small, it leads to random loss of allelic diversity and fixation of neutral alleles linked to selected ones[1]. These are known as "hitchhiker mutations"[1,4]. Some authors use the term "passenger" mutation to refer specifically to the phenomenon in the context of cancer biology[4,17]. For Barrett's esophagus, it was shown that, other than a series of advantageous mutations in p16 and p53, all further lesion expansions could be viewed as hitchhikers on p16 lesion clonal expansions[21].

Population bottlenecks, such as those that occur under physiological condition in the breast epithelium during the menstrual cycle, or under pathological conditions in inflammatory bowel disease and Barrett's esophagus, as well as mutations that occur early onthogenetically and generate large clones ("jackpots") are also instances of genetic drift as a mechanism of carcinogenesis[4].

CANCER STEM CELL HYPOTHESIS

The cancer stem cell hypothesis states that only a small fraction of cancer cells possess the potential for self-renewal and propagation[19]. It was originally elaborated by experimentation with transplants of leukemic cells and, despite its being considered as applicable to all cancers, is still fairly controversial. There is debate on whether CSCs are rare or frequent, whether their phenotypes are fixed, hierarchical or variable. The single feature that is a mandatory is the potential for endless self-renewal. Quantifying this propensity for self-renewal through xenotrans-plantation or gene-expression signatures is already used to evaluate prognosis in some cancers. It stands to reason that in the course of cancer progression there is selection geared at favoring cells with the best capacity for self-renewal, to the detriment of those who tend to differentiate. If these cells are the primary motor of cancer-clone evolution, it follows that any therapy should have its final goal CSC inhibition or destruction. The CSCs' genetic and epigenetic diversity are widely viewed as one of the main reasons for therapeutic failure[17]. Some researchers[19] view the cancer stem cell hypothesis as an alternative to somatic evolution as the putative central dogma of cancer biology.

IMPLICATIONS FOR CANCER THERAPIES

The phenomenon of acquired drug resistance, first attributed to somatic evolution by Nowell[3], has been studied for several decades. The oldest studies conducted involved chemotherapy agents. They identified the emergence of methotrexate resistance, which had arisen through amplification

of the dihydrofolate reductase gene upon which it exerts its effects[19, 22–25]. This was also found to be true of 5-flurouracil, which led to positive selection for cells in which its target gene thymidylate synthase was found in increased numbers[19, 26]. One of the most influential studies in this regard has been the one concerning chronic myeloid leukemia, in which blood samples from patients who had suffered relapses after sequential treatment with imatinib and dasatinib showed evolving resistant mutations in the BCR-ABL gene that induce resistance to sequential ABL kinase inhibitor therapies[19,27].

With acquired therapeutic resistance showcasing an evolutionary dynamic, the solution lies in the ability to design therapeutic interventions that simultaneously reduce tumor burden and retard the evolution of therapeutic resistance. Since resistance to different drugs arises most often from different mutations, it stands to reason that combination therapy has less chances of encountering cells with resistance than single agent therapy does, thereby reducing the likelihood of relapse.[19]. It has been shown that combination therapies have a better response rate, as well as reduce the rates of relapse, but this comes at the cost of greater toxiticity, and, moreover, mortality is only slightly reduced[19,28]. It can be therefore deduced that there is a trade-off between toxicity and obstructing resistance to treatment. Also, unlike in other condition where multidrug treatments have managed conversion to chronic illness (most notably HIV/AIDS), the same has not been achieved for cancer. While the precise causes have yet to be identified, mutations causing an increased activity of efflux pumps have been incriminated for multi-drug resistance[19].

THE QUASI-FAILURE OF TARGETED THERAPIES

One of the newest strategies for treating cancer, and one that has gained much attention in recent years from the researchers actively engaged with the problem of developing new cancer treatments is that of *targeted therapy*. In contrast to the traditional anticancer therapies that worked by stopping the growth of fast-dividing cells, with little or no selectivity for actual cancer cells, and which for that reason caused major and sometimes life-threatening adverse reactions, the promising targeted therapies consist of specifically engineered compounds and monoclonal antibodies directed against the very signal molecules that cancer cells use to communicate among themselves, induce and sustain growth and proliferation, coordinate migratory and invasive behavior[29]. The actual targets of these new drugs can be broadly divided into two main categories: the first comprises mutant proteins such as such as BCR-ABL, c-KIT, BRAF, and EGFR which are the aberrant products of malignant cells in some types of cancer, that actually promote the success of these tumors; the second type of targets concerns metabolic and signaling pathways that have become disregulated, and contribute to the pathogenesis of the cancer[29].

While it would stand to reason that such therapies would be an important step forward in reducing cancer mortality, thus far, for reasons insufficiently clarified at the moment, targeted therapies have not yielded the expected results. Cancer cells have been found to be much better at coming up with solutions to the pharmacological treatments they are faced with through targeted therapy, thus appearing to clinicians as having gained resistance to the treatment. This phenol-menon is as old as cancer treatment itself, and has recently come to the forefront of cancer research[30].

Given that mutation rates are extremely low for somatic cells[11], the question that arises is how such a diverse population of cells that neither combined chemotherapy nor the parallel efforts of the immune system can contain, so much so that in many cases it inexorably leads to the failure of the treatment and death of the organism. Thus, the question that ensues is whether the deterioration of the DNA repair machinery of the cell, also brought about by mutation, can be the sole inducer of such a morbidly prodigious variety of mutants, some of which get selected for by the pressure of the treatment, thus offering a mechanism for the phenomenon of tumor resistance that blunts the success of so many promising treatments[31], or whether the underlying mechanism is more complex than previously considered.

POTENTIAL THERAPEUTIC APPROACHES

Lambert et al. propose a novel way of looking at strategies and mechanisms used by tumor cells

for the progression of cancer. Rather that random mutation being the driver for generating genetic, and thus phenotypic, diversity in cancer cells[6], they posit that random genetic injuries to DNA are not sufficient to explain the behavior of tumor cells[32], but that cancer results from a deterministic and collective stress response[33] that is performed by interacting cells, which also maintain complex communication with the surrounding environment[32]. To overcome the technical difficulties faced by researchers trying to replicate *in vitro* the conditions occurring in human tissues, and more notably, inside different organizational layers of the tumor, the group has likened the process of evolving drug resistance that malignant cells exhibit to that by which bacterial communities gain resistance to antibiotics[32,34].

The mechanisms through which bacterial colonies gain a high degree of evolvability were examined, ascertaining that genetic instability which accounts for the evolvability of cancer cells does not arise from random mutations[32,35]. The concept of evolvability was employed by Sniegowski and Murphy to designate mechanisms bacteria use in order to promote evolution[35]. To gain further insight on cancer evolution, scientists have looked at microbial biofilms[36], complex structures used by bacteria under stress conditions to maintain a low penetrability environment for chemotherapeutic drugs. Within the microbial biofilm, bacteria retain a small mutator-phenotype population of cells in which gene clusters that account for protection against oxidative stress are down-regulated. In doing this, variants that are highly resistant to many of the common antibiotics are obtained[37]. Similarly, tumor cells co-opt stromal-vascular elements which then become part of the tumor mass and co-evolve alongside cancer cells[38,39]. Among these, fibroblasts are driven by molecular signals to increase extracellular matrix production[38], which in turn, acts as a microenvironment similar to the bacterial biofilm, providing a barrier against the diffusion of most drugs. The oxygen-deprived cancer cells are also sustained by close metabolic collaborations with the vascular-stromal elements[39]. These findings also raise the possibility of alternative therapeutic strategies, targeting the cancer cells' environmental habitat rather than cancer cells themselves. This is known as ecological therapy. Examples are anti-angiogenesis, interfering with bone remodeling in prostate cancer, aromatase inhibitors in breast cancer, amplifying hypoxia, as well as the blockage of the cells' interaction with vascular-stromal elements[17]. One of the main Darwinian advantages that ecological therapy poses is that the drug targets in this case exhibit minimal heritable variation in resistance. The variation between neoplastic micro-environments, as well as their number itself, is putatively much smaller than the number of cancer cells and their respective genetic variability[19]. This has been conclusively shown for anti-angiogenic drugs, which unlike standard chemotherapy, do not give rise to acquired drug resistance[40].

Gillies *et al.* also identify the two most important aspects that cancer research must take into account from an evolutionary standpoint: the selection pressure posed by the environment in which tumor cells find themselves, and the corresponding adaptive strategies they employ to evolve resistant clones. The article focuses on the time-dependent dynamic of cancer evolution. It posits that an evolutionary take on cancer therapies and their quasi-failure can hardly be overemphasized, and that this is the paradigm against which new research in the field should be judged. It attributes the failure to the fact that, thus far, researchers have mostly ignored the fact that the nature of the organism itself is a Darwinian one, subjected to the forces of natural selection, and for that matter, is is continuously able to adapt to external pressures[18].

An evolutionary understanding of cancer also points towards other potential therapeutic approaches. Two such strategies have shown great promise in computational models – benign cell boosters and chemosensitive boosters. Benign cell boosters aim to increase the fitness of the relatively benign cells that surround or are part of a neoplasm, with clonal competition driving less benign cells extinct and halting tumor growth as a consequence[8]. For example, proton pump inhibitors act in this manner in Barrett's esophagus and are currently used to inhibit acid reflux into the esophagus, thereby favoring the growth of normal squamous epithelium instead of the neoplastic one that would otherwise appear[19, 41]. Chemosensitive boosters are analogous, the aim being in this case increasing the fitness of chemosensitive cells, in order to outcompete the chemoresistant ones. At a later stage, when chemotherapy is commenced, all the tumoral cells will be sensitive to chemotherapy. This is known as the "Sucker's gambit". However, both these approaches are theoretical, to be used as benchmarks for exploring evolutionary strategies in cancer treatment and preventive medicine[8].

CONCLUSION

Nowell's hypothesis, according to which drug resistance in cancers is driven by somatic evolution[3], has been proven to backed by substantial evidence[19]. Cancers can be aptly described as microcosms of evolution[4]. Regarding cancer as subjected to evolutionary imperatives sheds light both on the reasons of its emergence, as well as on the setbacks that therapeutic efforts have encountered thus far[4]. There are two clinical issues that benefit most from the perspective offered by an evolutionary view of cancer – neoplastic progression and acquired therapeutic resistance[19]. An evolutionary view of cancer is paramount for drug development, as a potential solution to previous difficulties as well as a source of new approaches to the matter. While evolutionary biology is generally a descriptive science, evolution as applied to cancer biology must necessarily be an interventional one, seeking to manipulate the evolutionary process itself[19].

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