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REVIEW

Multidimensional Complexity of Cancer. Simple Solutions Are Needed

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Abstract—Cancer is a complex system. Tumor complexity is determined not only by genetic and epigenetic heterogeneity, but also by a huge number of interactions between cancer and normal cells. The heterogeneity and complexity of a tumor causes failure of molecular targeting therapy as a tool for fighting cancer. This review considers the concepts of malignant tumors as organisms that have common characteristics despite all heterogeneity. This leads to the idea that one of the most promising strategies for fighting cancer is the use of the patient's immune system.

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In 2009, *Biochemistry (Moscow)* published my analysis "Fundamental Taboos of Biology" [1], in which I stated, *inter alia*, that it was highly improbable to find two identical cells of complex eukaryotic organisms, both from the standpoint of genetic architecture and epigenetic factors, metabolic state, etc. This is especially true for cancer cells. Since that time, great progress has been made in the analysis of genome primary structures in general, and genomes and transcriptomes of cancer cells in particular. A major project devoted to sequencing of 10,000 tumor genomes was started in 2006 as The Cancer Genome Atlas (TCGA), valued at $100,000. It was officially finished in 2014, has given rise to The International Cancer Genome Consortium, and revealed about 10 million (!) mutations associated with cancer (traditionally, most studies were focused on cancer cells) [2]. But even this large number in no way reflects the whole complexity characteristic of cancer (for definition of complexity, see [3]). It turned out that heterogeneity among cancer cells is much higher than I could imagine in 2009 and even in 2011 [4].

It should be mentioned, however, that it is now believed that for the most widespread cancer types, only 140-200 of 20,000 genes in the human genome can be driver genes (that determine the fate of a cell). Mutations in driver genes of a given cancer type (driver mutations) are found in most patients [5]. The other (passenger) mutations arise accidentally in the process of the long evolution of cells to a cancer phenotype, their frequency of occurrence in a given cancer type is considerably lower, and, according to many, do not appreciably affect tumor evolution [5]. However, such strict division of mutations into "driver" and "passenger" should be taken with caution. "Passengers" can change into "drivers", and *vice versa*, depending on the conditions and genetic background of the tumor. I mentioned in 2011 [4] and further substantiated in a recent review [6]: "...it should be recognized that the distinction between driver and passenger mutations in a tumor may be a dynamic one, as the most advantageous (fittest) genotypes are not the same in all cancers or at all times or places in the same cancer, because the selective advantage of any genotype is dependent on the environment. The environment must be defined broadly to include a cell's repertoire of gene and protein expression, the mutations and epigenetic changes already present in that cell, the local external microenvironment, distantly acting factors (such as hormones), and factors external to the host (such as carcinogens and exogenous therapies). For example, late-stage tumors may not always rely on an early driver, etc."

The genome of each cancer cell has 10,000-20,000 mutations, which can provide about $10^{68,000}$ different cells [4]. In other words, no two identical cells exist in one tumor, or two identical cells in different tumors. This is

Abbreviations: CAFs, cancer-associated fibroblasts; CML, chronic myelogenous leukemia; EMT, epithelial–mesenchymal transition; MET, mesenchymal–epithelial transition; MF, myofibroblasts; MSCs, mesenchymal stem cells; TME, tumor microenvironment; Treg cells, regulatory T-cells.

also many times reinforced by the existence of epigenetic, metabolic, and other types of heterogeneity. The complexity of the tumor is not limited to genetic and epigenetic heterogeneity. The main problem is that cancer is a system belonging to the scientific category of "complex systems". A brief description of the hallmarks that characterize complex systems is given in the next section.

CANCER IS A COMPLEX SYSTEM WITH ALL PROPERTIES INTRINSIC TO COMPLEX SYSTEMS

A detailed examination of the problem of complex systems was presented in my recent review [3]. The last review with description of complex systems can be found in [7]. In this review, I give only a brief characteristic of the main points.

A complex system is a multicomponent system of *interacting* subunits, the interaction of which results in *emergent* properties inherent to the whole system and *not predictable* from the properties of the initial subunits. The famous geneticist Mayr (1998) wrote: "a description of the isolated parts fails to convey the properties of the system as a whole..." (quoted from [8]). The emergent properties are the most important feature of complex systems. They cannot be attributed to particular interacting components; this is a property of the whole system. Furthermore, a system may have a number of hierarchic levels, each of which has its own emergent properties [9-13]. Complex systems include different elements, the collective behavior of which can be characterized as "the edge of chaos" [9, 14, 15]. Complex systems are nonlinear and *extremely sensitive to initial conditions* [8]. It means that the trajectory of a system [9], defined, e.g. as a change in its condition in time, is not predictable. It refers to a change in such parameters as temperature, pressure, hemogram, etc., which determine the state of a patient in time. The dependence on initial conditions means that two systems in very similar initial states that function according to the same rules will have different trajectories. The immune system contains various elements (macrophages, T- and B-cells, etc.) that interact by the exchange of signals (in particular, cytokines). Even when exposed to exactly the same stimuli, the immune system may respond quite differently. Different behavior is determined and depends on the action of multiple internal and external factors [16]. Advanced technologies of observation and testing biological systems only led to further levels of complexity [8, 16]. Due to the above-mentioned features, complex systems as a whole cannot be simulated using computer modeling [8, 17].

The complex systems are nonlinear, that is their response to a sum of external signals is not equal to the sum of responses to all these signals taken separately [18]. Slight changes of some signals do not necessarily provoke similar slight responses of a system, and *vice versa*. An unexpectedly great effect in response to a slight action is quite common. Living systems usually evolve to the edge between order and disorder [9, 14, 15]. A complex system at the edge of chaos can demonstrate regular and predictable properties, but can also undergo unexpected massive stochastic changes in response to seemingly insignificant actions, signals, or stimuli [14, 15]. Many tissues, especially epithelium, are highly ordered, less prone to variations, and the behavior of their cells is quite predictable. However, emergence of genetic instability can cause cancer with a huge heterogeneity within the tumor, thus providing resistance to drugs. This is a result of the disturbance of complex adaptive systems of cell interactions that are important for regulation [19, 20]. The behavior of complex systems can hardly be predicted. To have exact predictions, one needs an infinitely exact and therefore impossible description of initial conditions [21]. For example, each cell is unpredictably different from another, both genetically and due to so-called genetic noise [4, 22]. It is impossible to predict what site of the genome will be mutated during a given cell division, and therefore it is impossible to predict the genome structure of a particular cell taken from the descendants of a precursor cell. It is impossible to exactly predict the influence of environmental factors (as well as the influence of stochastic factors) on a complex system, which starts *in utero* and continues during all the life of the individual [23].

A cancer tumor comprises a changing in time and space variety of cells, each of which has its own signal cascades, replication, transcription, etc., and undergoes multiple changes on the way of its transformation into a cancer cell. It acquires the complexity of a growing and evolving system [24] with all the features and properties that allow resisting anticancer drugs and providing intratumoral cell heterogeneity, making the tumor unique for each patient [25]. This property of cancer distinguishes it from all other diseases [24]. However, the complexity of the tumor is far not limited to sets of cancer genes or cells that in varying degree have an effect on the tumor progression. In their last version of cancer hallmarks, D. Hanahan and R. A. Weinberg [26] remark that tumors exhibit another dimension of complexity due to the recruitment of a wide spectrum of normal cells to their evolution and adapting them to their needs. These cells facilitate the acquirement of hallmarks, creating what is called the tumor "microenvironment" and its ecological niche, which plays a most important role both in the evolution of the primary tumor and its metastasis. Today, we can confidently believe that the main complexity of the tumor resides in a huge number of interactions between cancer cells (usually epithelial ones) and various stromal cells that form the tumor microenvironment (TME) [27].

There is one more level of complexity. It becomes more and more obvious that a tumor produces factors affecting various systems of the organism, and *vice versa*.

For example, changes in the functioning of the bone marrow can be observed. Distal hormonal signals and inflammatory mediators facilitate the formation of premetastatic niches where tumor cells anchor, exist in a dormant state, and finally transform into growing metastases. Blood and lymphatic vessels provide the delivery of nutrients to the tumor and link it to other parts of the organism. The tumor and its microenvironment produce many factors that affect metabolism of distant tissues and organs. This distant environment forms a macroenvironment constantly interacting with the tumor. Body weight loss provoked by cancer is the best-known syndrome of the interaction between the tumor and macroenvironment [28, 29]. Nevertheless, this brief review will be devoted to much better studied effects of the microenvironment.

MICROENVIRONMENT, A KEY ELEMENT OF TUMOR EXISTENCE AND EVOLUTION

A philosopher of science, Evelyn Fox Keller, entitled her remarkable book "The Mirage of a Space Between Nature and Nurture" [30], meaning that there is an inextricable link between genetically determined qualities and those determined by the environment. The closest environment of cancer cells is so-called tumor stroma or the tumor microenvironment composed of networks of lymphatic vessels, extracellular matrix, and various non-tumor cells, including stromal fibroblasts and blood cells, as well as (and this is essential) infiltrating immune cells. Regulatory T-cells (Treg cells) play a key role in malignant tumor progression and make a most important contribution to the resistance of the tumor to traditional therapies [31]. There is compelling evidence for mutually beneficial interaction of partially transformed cells with each other and with neighboring stromal cells, which is advantageous for progressing tumor cells [24, 32-34]. TME functions "as a double-edged sword"; it either facilitates tumor progression, or *vice versa*, exerts antitumor activity. It operates in a complex tangle of signals that are difficult to separate and experimentally characterize. The interaction of TME with a tumor is realized either via intercellular contacts or by exchange with soluble factors [35]. Lately, new key players of this interaction have been identified, such as secreted microRNAs, metabolites, and exosomes [36].

A tumor is often considered as a complex "organ", being composed of interacting highly heterogeneous cells and subcellular structures and representing a single entity that incorporates cancer and surrounding cells [37]. TME forms a barrier that protects the tumor from external influence, which includes cells of the immune system, and thus takes part in the creation of an immunosuppressive phenotype [38, 39]. It is the escape from immunological surveillance that is known to be one of the most important factors of tumor development in the organism [39]. An increase in the number of intratumoral, cancer-specific T-cells will be ineffective if, due to the obstacles created by stromal cells, T-cells are unable to accumulate in close proximity to cancer cells [39]. The available data clearly indicate that the tumor microenvironment distinctly differs from the corresponding normal stroma. Despite a more chaotic organization as compared to highly organized healthy tissues, cancer demonstrates strictly differentiated structures, synergy with, and dependence on penetrating connective tissues [40]. Accumulating data indicate that endothelial cells retain functionality during tumor development. Tumor vessels deliver and recruit mesenchymal stem cells (MSCs) to neoplastic tissues. MSCs together with resident normal fibroblasts acquire an activated phenotype and form cancer-associated fibroblasts (CAFs), the most essential element of tumor stroma. Microenvironment remodels the extracellular matrix and provokes the loss of smooth muscle cells and a continuous influx of myofibroblasts [41]. This by no means full list of interactions of cancer cells with the environment provides a glimpse of the complexity of a tumor system [40]. A particular problem related to the complexity of cancer is metastasis, to which a special issue of the journal *Science* (dated April 8, 2016) was devoted. Since concepts in this field of oncology are rapidly changing, a brief review cannot reflect, even to a small extent, the contradictions, directions of studies, findings, and gaps faced by researchers of metastasis.

Cancer is a co-evolving cell population, and an important feature of this co-evolution is transdifferentiation of progressing tumor cells. The tumor stroma contains fibroblasts, myofibroblasts (MFs), endothelial cells, and cells of inflammatory response associated with the immune system. The origin of MFs is not fully clear, but the data available suggest that the main source of tumor MFs might be the epithelial–mesenchymal transition (EMT) [42-44]. Myofibroblasts appearing in the tumor stroma can form and modify the extracellular matrix, secrete angiogenic and proinflammatory factors, and stimulate the division and invasion of epithelial cancer cells. The reverse mesenchymal–epithelial transition (MET) is also possible [42]. Both transitions are suggested to play a major role in metastasis. This is one more level of the complexity and heterogeneity of the developing tumor. Heterogeneity results in different initial conditions for a complex cancer system, which determines the unpredictability of its trajectory, and therefore the outcome of the therapy and disease.

INEVITABILITY OF TARGETED CANCER THERAPY FAILURES

Taking in mind this heterogeneity, I, as well as the other authors [45], predicted in my review [4] a high

probability of inefficiency of molecular targeted cancer therapy that is a therapy targeted at certain molecular components of a tumor. Further experiments have fully confirmed this prediction. The extreme tumor heterogeneity is of significant importance in clinic and causes a disparity between the cost and efficiency of anticancer-targeted approaches. For example, in the period between 2002 and 2014, the US Food and Drug Administration approved 71 anticancer therapeutics, and 52 of these were targeted agents. These gave an increase in the median overall survival of as little as 2.1 month (!) at a cost of $2.7 million per life-year saved [46]. Targeted therapy can probably reach maximum efficiency only for targets that are present in all cancer cells [47]. Moreover, there is more and more evidence that even before the start of the therapy, polygenic mechanisms of drug resistance in subclones exist [46]. In recent years, the general crisis of efficiency of the pharmaceutical industry has been widely discussed (e.g. see [48, 49]). As an example, Stock et al. wrote: "These levels of failure question the effectiveness of the reductionist [molecular] target-based paradigm that has become the mainstay within the pharmaceutical industry and implies that radical improvements are required in the selection of disease-relevant therapeutic targets" [50]. The first and the most representative example of targeted therapy is the anticancer drug Imatinib successfully used for treatment of chronic myelogenous leukemia (CML). However, CML may be unique in that it is determined by a single molecular anomaly, the Philadelphia chromosome containing the *BCR-ABL* gene, while anomalies subject to targeted therapy in most other cases of cancer are numerous. The Philadelphia chromosome was observed in 90% of CML cases. Many researchers believe that Imatinib is an exception rather than the rule, and therefore its success leads us in a wrong direction [51]. Failures of targeted therapy are inevitable due to the limitations mostly resulting from the absence of a proper target for known targeted agents in most oncology patients.

The well-known Nobel Prize winner and enthusiast of targeted therapy, James Watson, stated the following ideas in his interview to Alla Wagstaff [52] (as a quote from her paper): "Now Watson [who was the first director of the Human Genome Project] ... is questioning whether genetic approaches to treating cancer can ever lead to the breakthroughs we need. At 85 years old, Watson has spent recent years applying his vast knowledge and impressive intellect to the problem of incurable cancers and has reached the following conclusions: To cure cancer you need to kill cancer cells. Targeted biological therapies do not kill cancer cells, they are not curing cancer, and it is unlikely that they can be made to do so in a practical or comprehensive way in the near future. It is time for a change in strategy. We know the current approach is not working...". In addition, here is a typical quote from a paper of an ordinary researcher working in this field: "The promise to understand cancer and develop efficacious therapies by sequencing thousands of cancers has not occurred. Mutations in specific genes termed oncogenes and tumor suppressor genes are extremely heterogeneous amongst the same type of cancer as well as between cancers... Is it time for a new approach to understanding and ultimately treating cancer?" [53].

FUNDAMENTAL CRISIS OF REPRODUCIBILITY

The complexity of cancer has a number of important practical consequences. I will only dwell on one, but an extremely disturbing one of them. Scientists of the biotechnology company Amgen, California, tried to confirm published findings related to their work. Fifty-three papers were taken for examination, and scientific findings were confirmed in only six (11%) cases. Even knowing the limitations of preclinical research, this was a shocking result [54, 55]. Taking into account this result, a project aimed at testing reproducibility of individual published reports on new discoveries in cancer research was initiated. This project was published in June 2015 in the journal *Science* [55] (see also Reproducibility Initiative, http://www.elsevier.com/connect/seeking-certainty-why-the-results-of-50-landmark-cancer-studies-are-being-examined). It should be noted that the project did not enthuse the researchers who were to voluntarily provide their findings for tests of reproducibility. They can be understood: if even the results are reproduced, they most probably will be reproduced only in part.

Further, I will try to express the idea that the main cause of the irreproducibility is the extreme variability characteristic of biological systems in general, and increased manifold in the case of cancer. But first I would like to acquaint the readers with the analytical review of C. Begley and J. Ioannidis published in 2015 and devoted to the situation with reproducibility of biomedical data [56]. The review is entitled "Reproducibility in science: improving the standard for basic and preclinical research". Here is what the authors, well-known specialists in the field of statistical treatment of experimental results, wrote: "Over the recent years, there has been increasing recognition of the weaknesses that pervade our current system of basic and preclinical research. This has been highlighted empirically in preclinical research by the inability to replicate the majority of findings presented in high-profile journals. The estimates for irreproducibility based on these empirical observations range from 75 to 90%. These estimates fit remarkably well with estimates of 85% for the proportion of biomedical research that is wasted at-large. This irreproducibility is not unique to preclinical studies. It is seen across the spectrum of biomedical research. For example, similar concerns have been expressed for observational research where zero of 52 predictions from observational studies

were confirmed in randomized clinical trials". The authors explain the problem: "At the heart of this irreproducibility lie some common, fundamental flaws in currently adopted research practices. Although disappointing, this experience should probably not be surprising, and it is what one would expect also theoretically for many biomedical research fields based on how research efforts are conducted" [56]. However, I would like to note that there are two sides to irreproducibility: an obligatory one, inevitably inherent to a system, and a methodological one due to drawbacks of the analysis methods. The latter may be also inevitable either because of the current absence of available analysis methods adequate for a given system, or poor planning of the experiment. The inherent irreproducibility can be due to the extreme variability of biological systems. It is practically impossible to find two identical cancer cells. This means that even true findings from one study need not to be necessarily reproduced in other studies [57]. The problem of irreproducibility troubles researchers very much. In 2015, the journal *Nature* published an issue "Nature special" devoted to problems of irreproducibility (http://www.nature.com/news/reproducibility-1.17552).

CANCER IS NOT WHAT WE THINK OF IT

Every member of the human population contains approximately 10^{13}-10^{14} cells, 70% of which are cells of symbiotic bacteria predominantly localized in the gastrointestinal tract. The human organism contains about 210 different cell types, 25,000-35,000 genes, about 10 million different proteins, 2000-3000 different low molecular weight metabolites. In addition, each human cell is made up of 10^{14} water molecules, $5 \cdot 10^{12}$ molecules of carbohydrates, $6 \cdot 10^{10}$ RNA molecules, $2 \cdot 10^{12}$ molecules of fats, and almost two meters length of DNA. The brain of an adult man contains up to $\sim 10^{11}$ neurons that form a synaptic network of 10^{14}-10^{15} synapses [58]. In addition, every individual represents a unique mosaic of cells that differ from each other genetically, epigenetically, and metabolically [1], and is different from other individuals in billions of molecular and cellular characteristics. In spite of this incredible molecular and cellular complexity, one can easily distinguish a man from an elephant, whose skin hides a similar complexity. The phenotype of an animal is much more stable than its genotype and associated molecular organization. One of the major unsolved problems of evolutionary genetics is why some traits are phenotypically invariant despite obvious genetic and ecological changes.

In 1942, Conrad Hal Waddington introduced the concept and term "canalization" to describe the robustness of phenotypes to perturbations in organism development. Canalization suggests that the phenotype of a given genotype remains relatively invariant and insensitive to differences in the environment (environmental canalization) and genotype (genetic canalization). Canalization results in the accumulation of phenotypically cryptic genetic variation, which can be used under specific conditions. Phenotypes that are identical and equal from the viewpoint of selection can arise from essentially different genotypes [59-61]. In changing conditions, selection can use this recently reacquired and capable of expression genetic variation. The accumulation of cryptic genetic variation due to canalization can increase the evolutionary ability of populations. Canalization, for example, can lead to genetic redundancy, modular structure of the organism, and new properties of genetic networks and biochemical pathways. Moreover, there is a hypothesis of positive correlation between the complexity of a biological system and its stability, defined as robustness against destructive effects [62]. Despite the beauty of this hypothesis, it still cannot be considered as definitely proven [61]. Lately, many attempts have been made to mathematically simulate this phenomenon (e.g. see [63-65]).

The concept of hallmarks of cancer [26] can be considered from the viewpoint of the canalization hypothesis. The hallmark concept suggests that the complexities of neoplastic diseases can be united under a limited number of hallmarks: (i) sustaining proliferative signaling; (ii) evading growth suppressors, resisting cell death; (iii) enabling replicative immortality; (iv) inducing angiogenesis and activating invasion and metastasis; (v) reprogramming of cellular energy metabolism ("Warburg-effect"); and (vi) evading immune destruction [26]. To this can be added one more very important hallmark, extreme intratumoral heterogeneity that makes the tumor resistant to various therapeutic actions. As mentioned above, the tumor has another aspect of complexity: apart from cancer cells, it contains a wide spectrum of recruited, seemingly normal cells that create the tumor microenvironment capable of considerably affecting the tumor characteristics. The other important factor, used by the tumor both to resist external effects and for metastasis, is an extraordinary plasticity leading to the epithelial–mesenchymal and mesenchymal–epithelial transitions. These transitions are used at most stages of the invasive–metastatic cascade. In turn, taking particular hallmarks as an integral system, cancer can be considered a single and developing organism, and therapeutic efforts should then be directed at the eradication of this organism as a whole.

Such ideas have been repeatedly expressed in the literature. Below are some examples. The authors of a work published in 2016 in the *BioEssays* journal wrote [66]: "Despite important differences between infectious diseases and cancers, tumor development (neoplasia) can nonetheless be closely compared to infectious disease because of the similarity of their effects on the body". The authors believe [66] that many regularities of the development, characteristic of host–parasite relations, can also

be characteristic of cancer (see also [67]). Vincent [68] suggested considering cancer as a revival of a programmed and evolutionary conserved form of life that appeared and developed in the Precambrian, rather than simply a series of random mutations. The central position in this program is occupied by the Warburg effect and genomic instability, which are supposed to be the main targets of therapeutic action. The same group further develops this idea towards the elaboration of a therapeutic strategy aimed primarily at the induction of the host immune response [69]. The group of Duesberg suggests that "cancers could be species of their own, and carcinogenesis could be a form of speciation" [70].

Naturally, those authors speculate on what type of therapy follows from the acceptance of this speciation theory, and various alternatives are considered. Nonetheless, most researchers prefer the strategy of the induction of the host immune response taking into account the peculiarities of cancer species. It is not easy, but immunology often helped humanity in critical situations, even before it got to know about viruses and bacteria. Works in this direction are currently intensively ongoing. Here, encouraging results, obtained in the immunological fight against metastases, should be especially noted. The interested reader can find these results in the abovementioned issue of *Science*. The present review can be finished with the concluding sentence of the introduction to this issue: "Nonetheless, there are reasons to be hopeful. Immune-based therapies ... are showing promise in preclinical models. Perhaps one day the research inspired by awe will stamp out fear [of cancer and metastases]" [71].

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