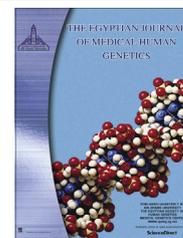




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REVIEW

Cancer: Some genetic considerations



Mohammad Saad Zaghloul Salem

Faculty of Medicine, Ain-Shams University, Cairo, Egypt

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- Temporal imprinting;
- Malignant transformation;
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- Evolutionary paradox;
- Metastasis;
- Oncoproteome;
- Cancer therapy;
- Oncogenome

Abstract Malignant transformation of normal cells to cancer cells represents an enigmatic phenomenon because of the many ambiguous controversies embodied within most of its aspects. Within a clinical context, cancer, with very few exceptions, is a dreadful disease that ends lethally. Within a biological context, however, cancer is a peculiar biosystem that has its own rules that regulate the actions/interactions/structure and behavior of its components. Unfortunately, the majority of these rules are, still, unknown.

The current disappointing situation as regards research trials aiming at constructing effective treatments for cancer might be attributed, in part, to incomplete recognition of the significant differences between these two contexts of malignant transformation. Although the peculiar characteristics of cancer as a self-dependent biosystem are well studied and well defined, the basic dilemma of malignant transformation continues to exist: we know, largely, how things happen but we do not know, to any extent, why they happen.

Though the logic that motivates researches aiming at formulating genetic therapies for cancer is quite reasonable, as cancer is primarily a genetic alteration, lack of essential basic knowledge regarding the different aspects of this alteration adjourn successful radical cure of cancer. Till comprehensive disclosure of the underlying mechanisms regulating growth/progression/metastasis and survival of malignant cells is attained, treatments of cancer based on different strategic concepts, viz. proteomic therapies rather than genetic therapies, might, hopefully, be the best approaches available in the fight against cancer in the current as well as in the coming era.

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Contents

1. Nature of malignant transformation	2
2. Genomic reprogramming of cancer cell	2
3. Genomic involution and hypodiploidy in cancer	2
4. Stem cell origin of cancer	3
5. Comparative genetics of tumors in humans, animals and plants.	3
6. Metastasis of malignant cells	4
7. Immune responses against cancer	4

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8. Genetic imprinting defects in malignant transformation	5
9. The evolutionary paradox of cancer	6
10. Clonal origin of tumors	6
11. Genetic therapies of cancer	7
12. Conclusions	9
Conflict of interest	10
References	10

1. Nature of malignant transformation

Malignant transformation of normal cells to cancer cells represents a radical change in the predefined default programming of the genome. The fate of normal cells is precisely defined according to a dogma that specifies the course of life at the molecular level. The genome of the cell dictates the basic characteristics of the cell within the context of the cell population, tissue or organ as regards essential aspects including growth and differentiation, timing of cell division, synthesis of products needed for mediating physiological functions, interactions with adjacent cells, responses to extracellular stimuli and regulatory mechanisms, and, most important, cell death when the mutation load of the cell causes considerable deterioration of cellular functions and imposes on the cell overburden pressure that drives it into apoptosis.

Transformation of normal cells to malignant cells implies numerous changes involving two main aspects of the cell: cell functions and cell architecture. Functional changes imposed by malignant transformation present as loss of functions, acquisition of new functions and quantitative/qualitative changes of preserved functions. Changes of cell architecture induced by malignant transformation impart to the cell new morphological characteristics that differ greatly from the structural properties of normal cells, and play a detrimental role in defining the natural history of tumor progression and metastasis. The marked deviation of the newly acquired structural and functional characteristics of cancer cells from normal cells constitutes the framework of the malignant phenotype which characterizes each type of malignant tumors.

The marked similarity of the phenotype of malignant cells to that of early embryonic and fetal cells represents an essential clue to understand and interpret the nature of the genetic alterations involved in the process of malignant transformation. This similarity comprises the general cardinal properties of tumor cells including enhanced rate of cell division, mass expression/suppression of large number of genes, resilience and plasticity of the cell cytoskeleton allowing for cell dissemination/migration and metastasis, augmented potential of differentiation/specialization/growth and, most importantly, altered pathways of apoptosis which allow for longer survival of cancer cells with consequent potentiation of the functional profile of the malignant phenotype. This hazardous result of halted or reduced apoptosis in malignant cells plays a critical role in conferring the aggressive behavior upon the cells and in maintaining tumor growth/progression and metastasis, which is the main culprit responsible for the dreadful end of cancer patients.

2. Genomic reprogramming of cancer cell

The nature of the malignant transformation of normal to cancer cells is still very far from being completely revealed or

properly understood. Many perplexing phenomena of this transformation have no interpretation largely because of lack of sufficient information regarding the underlying mechanisms involved in this mysterious biological behavior of cells. For instance, though some aspects of the malignant phenotype impart many selective advantages to the malignant cell, on the whole cancer represents the most disadvantageous fate of the genetic material that initiates and maintains this phenotype. In direct contradiction to basic concepts of biological evolution, the selective advantages conferred upon the cell by the malignant phenotype paradoxically result in self destruction and final extinction, rather than preservation, of the genome.

Reversion to the early embryonic/fetal state is the most remarkable genomic alteration that characterizes the malignant phenotype. This radical change is reflected, not only in altered cell functions and cell morphology, but also in the behavior of many types of tumors that aim at formation and establishment of a new creature, like malignant teratomas. To a lesser degree, the incongruous formation of incompletely differentiated tissues and incomplete/malformed parts of organs, and the apparently haphazardous transcription and synthesis of products, RNA/proteins/hormones/enzymes/etc., by metastatic tumors, might be considered within the same context. These observations might suggest the preservation of the evolutionary ability of the zygote to develop into a fully-developed organism, by descendant cells, particularly malignant cells. This reversion to the initial original genetic profile suggests the maintenance of the structural and functional phenotype of differentiated normal cells by genomic regulatory mechanisms controlled by master genes, probably through synthesis of mass silencers or suppressor molecules capable of keeping the rest of functionally unneeded genes in differentiated cells in a suppressed state. Disruption of these regulatory mechanisms would result in cessation and removal of mass suppression, with consequent mass reactivation, of non-functioning genes. It might also result in suppression of already functioning genes. These contrasting genetic alterations reflect and represent the actual *reprogramming*, or more accurately *deprogramming*, process of the genome of normal cells that, probably, paves the way toward the transformation to cancer cells and the establishment/initiation and progression of the malignant phenotype.

3. Genomic involution and hypodiploidy in cancer

In view of the complexity of the myriad of interconnected and interacting regulatory mechanisms that control and determine all structural and functional aspects of the cell, it is very difficult to accept the monoclonal theory of oncogenesis that attributes tumor development to a triggering mutational event in

one single cell. *First*, because these regulatory mechanisms are mediated by large numbers of proteins synthesized by large numbers of genes, and mutations affecting one or even few genes implicated in carcinogenesis, e.g., tumor suppressor genes/proto-oncogenes, cannot have such an extensive global effect on the integrity and stability of the genome unless they affect critical segments of the genome responsible for global regulation of the rest of the genome. Unfortunately, no such critical segments have been defined or, even, delineated. *Second*, most mutations have detrimental effects on one or more aspects of cellular functions, especially those mutations affecting cancer related genes, most of which are responsible for, and involved in, regulating the cell cycle. Accordingly, such mutational events leading to unexpected paradoxical effects that confer selective advantages upon the mutant cancer cell, like enhanced rate of cell division/prolonged survival and acquisition of new regenerative abilities, are better considered within a different context. *Third*, many types of cancer have astonishing, marked degrees of hypodiploidy, and to a lesser extent, hyperdiploidy. The ability of some malignant cells with as few number of chromosomes, as thirty chromosomes only, to retain their fully developed malignant phenotype and maintain their aggressive behavior in spite of loss of a considerable part of the genome, with consequent absence of a sizeable portion of the proteome, raises many queries regarding the nature of the underlying mechanisms involved in karyotypic evolution in cancer cells, and the nature of the possible genomic alterations that might compensate for such a conspicuous diminution of the genome size and the concomitant decrease in the transcriptome/proteome products.

The sustainment of the malignant phenotype in spite of marked hypodiploidy might be interpretable by many theoretical hypotheses. For instance, loss, or abandonment, of many non-vital or crucial functions of the differentiated normal cell upon undergoing malignant transformation would allow the cell to survive in absence of genes that regulate these functions. Over expression of the hypodiploid genome with sufficient near-threshold transcription/synthesis and/or partial replacement of deficient products might also allow for survival of malignant cells with marked hypodiploidy. An additional theoretical hypothesis entails functional complementation between different clones/strains/progenies of malignant cells within the tumor that have varying degrees of chromosomal hypodiploidy, with distinctive functional profile of each.

4. Stem cell origin of cancer

Many hypotheses regarding the origin of tumors from adult stem cells have been postulated based, mainly, on the striking parallels that can be found between stem cells and cancer cells as regards self-renewal property [1]. Later identification of cancer stem cells (CSCs); cancer cells found within solid tumors and hematological malignancies that possess characteristics associated with normal stem cells specifically the ability to give rise to all cell types found in a particular cancer sample, added support to this hypothesis. Furthermore, cancer stem cells are considered to be significantly responsible for growth, metastasis, invasion and recurrence of all cancers [2]. These postulations attributing carcinogenesis to be evoked by transformation changes of stem cells would be more reasonable than traditional postulations attributing it to mutational

events of proto-oncogenes or tumor suppressor genes of normal cells. The reason is obvious as induction of reversion of stem cells, programed for ready transformation to pluripotent/multipotent cells with phenotypic properties shared by cancer cells, by few mutational or reprogramming processes of the genome is more rational than attributing this radical and conspicuous alteration of the genetic constitution of the cell to one or few carcinogenic mutational events affecting cancer-related genes.

5. Comparative genetics of tumors in humans, animals and plants

Carcinogenesis and tumor formation in animals seem to be initiated and regulated by the same mechanisms responsible for tumor development in humans. Shared parallels between human and animal cancers comprise the main functional properties and morphological features that constitute the malignant phenotype and include mutations of specific oncogenes and/or tumor suppressor genes, altered pathways of apoptosis, markedly increased proliferation indices, defective DNA repair, modification of inter cellular connections and adhesion molecules, induction of angiogenesis, epithelial-mesenchymal transition and metastasis to distant sites. The great similarity between mechanisms of oncogenesis in humans and animals is expected in view of the shared genetic biosystems between both of them.

Contrary to what might be thought of, tumors are among the most widespread abnormalities of plant morphogenesis. Plant tumors, seem to be initiated and regulated by mechanisms different from those of human and animal tumors. In addition, they have unique characteristics. First, they might be caused by infection with viruses, bacteria, arthropods and worms, with crown-gall tumors caused by the bacterium *Agrobacterium tumefaciens* being the most common. Second, tumors may be formed in plants with specific genotypes and are referred to as genetic tumors. Plant tumors forming upon infection with *A. tumefaciens* are caused by conjugative transfer of DNA segment (T-DNA) from the bacterial tumor-inducing (Ti) plasmid, and in most dicotyledonous and some monocotyledonous plants malignant transformation leading to abnormal division/defective differentiation results from alteration in hormonal level or changes in hormone sensitivity induced by the activity of the plasmid genes integrated with the infected plant genome [3].

Although pathogenetic mechanisms underlying tumor formation in plants are poorly studied, currently defined functional aspects of plant hormones might have important implications for cancer therapy in humans. The leading role played by hormones in tumor development in plants is attributed to their global regulatory functions that control, nearly, all aspects of plant life. Plants, unlike animals, lack glands that produce and secrete hormones, instead, each plant cell is capable of producing hormones. Unlike specific physiological functions of hormones in human/animal cells, plant hormones, or phytohormones, regulate cellular processes in targeted cells locally and, when moved to other locations, in other locations of the plant. Plant hormones determine the formation of flowers, stems, leaves, the shedding of leaves, and the development and ripening of fruit. They also shape the plant, affecting seed growth, time of flowering, the sex of flowers, senescence of leaves, and fruits. They affect which tissues grow upward

and which grow downward, leaf formation and stem growth, fruit development and ripening, plant longevity, and even plant death. Hormones are vital to plant growth, and, lacking them, plant cells could neither grow nor differentiate, and plant hormones play pivotal regulatory roles in activating cellular responses, including cell death, to diverse stress situations in plants. Five major classes of plant hormones, some of which are made up of many different chemicals that can vary in structure from one plant to the next have been defined including Abscisic acid, Auxins, Cytokinins, Ethylene and Gibberellins. Other hormones with varying functions, e.g., growth regulators, signaling molecules, defense mediators etc., include Salicylic acid, Brassinosteroids, Jasmonates, Nitric oxide, Karrikins and many others [4].

The importance of plant hormones stemmed from observations regarding their effects on human cancer. For example, sodium salicylate has been found to suppress proliferation of lymphoblastic leukemia, prostate, breast, and melanoma human cancer cells [5], Jasmonic acid has been found to induce death in lymphoblastic leukemia cells, and methyl jasmonate has been found to induce apoptosis and cell death in a number of cancer cell lines [6]. Many researches have revealed the anticancer/antiviral/antiproliferative actions of many plant hormones, e.g., Brassinosteroids [7] and the antitumor and antiangiogenic effects of gibberellin derivatives [8]. In view of the marked functional versatility of plant hormones in controlling nearly all aspects of plant life particularly growth potential and proliferative properties, they may potentially constitute a novel class of effective anti-cancer medications.

6. Metastasis of malignant cells

Metastasis of malignant cells might be regulated by mechanisms similar to those regulating movement and decentralization of bacterial masses, e.g., quorum sensing pathways [9]. Furthermore, the positive feedback loop of this pathway including the coordinated synthesis and secretion of the signaling molecules (inducers) and the synthesis of the receptors with cumulative increase in size of population of the bacteria bears many similarities to the behavior of metastatic malignant cells.

The recent discovery of one isoform of the tumor suppressor gene p53 (p53 ψ) that may contribute to, rather than prevent, tumorigenesis proposed that increased levels of p53 ψ will enhance tumor development and aggression by sustaining the expansion and survival of putative cancer-initiating cells [10]. This proposed mechanism of the p53 ψ product permits speculations as regards a possible role of this gene in synthesis of inducer molecules that might have functional roles as signaling stimuli in the postulated quorum sensing pathway regulating some aspects of tumor metastasis.

Further support to this theoretical speculation regarding presumptive role of quorum sensing pathways in tumor metastasis might be deduced from the observation that synchronization of the behavior of hundreds of the bacterium *Myxococcus xanthus* cells in a growing swarm is, possibly, mediated by transmission signals. The way such large numbers of cells expand, align to each other and expand to form mounds or heaps under the effect of signaling/transmission molecules reveals, at least partially, how multicellular structures can be constructed according to an inherited plan [11].

The nature of the pathogenetic mechanisms underlying and mediating metastasis of malignant cells is poorly understood in spite of tremendous researches aiming at revealing pivotal aspects of this enigmatic behavior responsible for the lethality of the vast majority of cancers. Though many leading observations regarding this behavior have been considered, a common basic link between these findings has not been formulated. Selective genomic reactivation/suppression of genes responsible for regulating cell movement, cytoskeleton modifications and intercellular adhesions resulting in reversion to the early embryonic/fetal stage where cell migration to, and localization in, distant organs plays a crucial role in organogenesis, undoubtedly, has a central regulatory role in tumor metastasis.

Phenotypic alterations of cancer cells necessary for regaining this property are reflected in their ability to regain and undergo epithelial–mesenchymal transition (EMT), a transdifferentiation developmental regulatory process involved in early embryonic development resulting in disruption of homeostasis of embryonic/fetal epithelial cell and leading to acquisition of a migratory mesenchymal phenotype responsible for conducting critical developmental processes. This process is considered a crucial mechanism in tumor metastasis. The importance of cellular transitions in development is first apparent during gastrulation when the process of epithelial to mesenchymal transition transforms polarized epithelial cells into migratory mesenchymal cells that constitute the embryonic and extraembryonic mesoderm [12].

From the above mentioned observations, it seems plausible to postulate that metastasis of tumor cells might begin and progress along a strictly regulated multistage process comprising many genetic alterations of the genome/transcriptome/proteome compartments of the cell. This multistage process comprises many steps. First, reversion of cells undergoing malignant transformation to the embryonic/fetal cell stage with regaining the ability to undergo epithelial–mesenchymal transition. Second, synthesis of signaling factors and receptor molecules that predispose malignant cells destined for metastasis to distant locations, to respond and behave in a way similar to quorum sensing group behavior of bacteria. Third, establishment of metastatic niche in distant organs, preceded by, and/or followed by, widespread genetic alterations necessary for adaptation of metastatic cells to their new microenvironment. Proper interpretation of the different aspects of metastasis, however, awaits complete understanding of the predisposing genetic alterations and the actual pathogenetic mechanisms responsible for initiating and mediating this process.

7. Immune responses against cancer

The development of immune responses against tumor cells represents a perplexing phenomenon in biology of cancer. Though this process, considerably, occupies the focus of researches trying to design immunotherapies against cancer, lack of sufficient information about the actual pathogenetic mechanisms underlying its development hinders research approaches to design effective drugs in this respect. Since immune responses develop against foreign or nonself cells or molecules, their development against cancer cells in the body raises many queries regarding the true nature of immune responses developing against self compartments. Many

hypotheses have been postulated trying to interpret the actual causes of this altered behavior of the immune system, but none of them is satisfactory. For instance, the malignant phenotype may cause surface exposure of protein molecules that normally reside in, or are located inside, different cell compartments, e.g., cell nucleus/cytoplasm/intracellular organelles/etc., and that are not exposed to the effector cells/humoral networks of the immune system in normal conditions. Aberrant expression of tumor-specific antigens or of tumor-associated antigens either on cancer cell surface or in intercellular compartments/micro-environment of tumors will result in initiation of immune responses against developing cancer.

Synthesis by tumor cells of novel proteins capable of inciting immune responses might be possible under certain conditions. Infection with oncogenic viruses followed by integration of viral genome with specific sequences of target genes will result in synthesis of viral proteins or novel proteins translated by newly formed combinations of viral and host genomes. Genomic rearrangements caused by deleterious pathogenetic mechanisms, e.g., chromosomal breakage/translocations, might also result in the formation of new transcribing sequences within functional regions of genes with consequent synthesis of new proteins. Point mutations leading to synthesis of structurally defective proteins, e.g., wrong/truncated/longer proteins by mis-sense/non-sense/re-sense mutations respectively, and small mutations, deletions/duplications/inversions, can result in creation of new transcribing sequences leading to synthesis of new proteins which are defective, in most instances, due to defective or deficient post-translation structural modification, or more significantly, that might be located at wrong intracellular/intercellular sites or on the cell surface due to defective post-translation trafficking and localization of synthesized proteins. A different mechanism for attacking cancer cells by own body immune system comprises synthesis by tumor cells of proteins capable of neutralizing the effects of the humoral immune system and/or attacking or binding to immune cells responsible for recognition of, and differentiation between, self and nonself, thus nullifying their ability to recognize cancer cells or oncoproteins as foreign nonself components.

In spite of the major role of the immune system as the main defense mechanism against infection by different pathogens, viruses/bacteria/fungi/worms/etc., it plays a minimal role, if ever, in defending the body against development of malignant tumors. The reasons of failure of the immune system in combating cancer are many, and include ability of tumors to evade the immune system, reduced synthesis of MHC class I molecules on tumor cell surface thus avoiding detection by killer T cells, synthesis by tumor cells of protein products that inhibit the immune response; for example the cytokine TGF- β which suppresses the activity of macrophages and lymphocytes and development of immune tolerance against tumor antigens. Paradoxically, macrophages can promote tumor growth when tumor cells secrete cytokines that attract macrophages, which then generate cytokines and growth factors that enhance tumor growth and metastasis [13]. These disappointing findings indicate that immunotherapeutic approaches, e.g., cell-based therapies, antibody therapies and cytokine therapies, for treatment of cancer would have minimal impact in this regard.

The assumption that chronic non-infective inflammation with concomitant activation of the immune system may lead

to development of malignancy has been postulated in view of a number of findings. First, the inflammatory process itself provides the prerequisite environment for the development of malignancy as it includes upregulation of mediators of the inflammatory response such as cyclo-oxygenase (COX)-2 leading to the production of inflammatory cytokines and prostaglandins which themselves may suppress cell mediated immune responses and promote angiogenesis. These factors may also impact on cell growth and survival signaling pathways resulting in induction of cell proliferation and inhibition of apoptosis. Furthermore, chronic inflammation may lead to the production of reactive oxygen species and metabolites such as malon-di-aldehyde within the affected cells that may in turn induce DNA damage and mutations and, as a result, be carcinogenic. This assumption proposes that the conditions provided by a chronic inflammatory environment are so essential for the progression of the neoplastic process that therapeutic intervention aimed at inhibiting inflammation, reducing angiogenesis and stimulating cell mediated immune responses may have a major role in reducing the incidence of common cancers [14]. Criticism of this assumption rests on many opposing findings headed by the fact that the vast majority of tumors develop without a preceding inflammatory condition. Additionally, the chronic inflammation might itself represent immune responses to early cellular alterations reflecting ongoing malignant transformation, rather than a causative factor responsible for tumor development and progression.

8. Genetic imprinting defects in malignant transformation

Following completion of embryogenesis/differentiation/specialization/development/growth and formation of a fully organized fetus, the vast majority of genes in somatic cells get functionally imprinted as inactivated genes, only genes needed for mediating cellular functions of the cell are kept active. Large numbers of genes are known to control and regulate different aspects of post-fertilization processes and promote the exceedingly accelerated rate of growth and proliferation of embryonic and fetal cells. As the need for the functions of these genes diminishes markedly after that, these genes get suppressed. This type of time-defined imprinting, or temporal imprinting, is different from location-defined, or spatial imprinting, where genes become suppressed or activated by effects exerted by adjacent chromatin networks, and also from parent of origin effect, or parental imprinting, where genes remain active or get silenced according to the male or female parent they came from. The traditionally held view that some genes are imprinted applies only to genes affected by parental imprinting, whereas the majority of genes in somatic cells are temporally imprinted.

Though identical single or multiple mutational events affecting the same proto-oncogenes/tumor suppressor genes in a group of cells can theoretically predispose to malignant transformation of large number of cells at the same time, synthesis of one or few oncoproteins leading to disturbance of one or few metabolic networks seems quite insufficient to drive the cell environment, comprising thousands of networks and much larger numbers of proteins, to a malignant phenotype. Defects in maintaining temporal imprinting status of genes, which have key roles in early development and that become functionally silenced or suppressed after differentiation and development

are completed, might offer another plausible hypothesis to explain some aspects of initiation of malignant transformation. For example, it can offer a reasonable interpretation to the postulated reprogramming/deprogramming processes of critical centers/regions of the genome that probably initiate the early alterations/transformations in malignant cells. Additionally, it might also offer a conceivable interpretation to the possible pathogenetic mechanisms underlying the development/maintenance and progression of the malignant phenotype as well as the mechanisms of loss and/or acquisition of many structural features and functional properties of malignant cells based on the genomic reversion assumption.

9. The evolutionary paradox of cancer

Revealing the nature and the significance of the pathogenetic mechanisms underlying the acquisition of the different morphological features and functional properties of the malignant phenotype is necessary for proper understanding of the key steps in carcinogenesis in their temporal order. Still unexplained radical widespread reprogramming/deprogramming of the genome heralds the first step toward establishment of a new/different functional framework of the basic life constituents of the cell, the genome/the transcriptome/the proteome. Concomitant crucial changes of the transcriptome profile follow and result in extensive reframing and delineation of most functional/structural networks of the cell, metabolic networks/signaling networks/growth-proliferation networks/cell migration networks/etc., mediated by the new/altered proteome profile of the cell. Each newly constructed/different network comprising the newly synthesized proteins, in addition to original proteins as well, begins to confer its novel regulatory functions upon cell constituents involved in performing these functions. This sequence of events results in establishment of a new phenotype at the molecular level as well as on the cellular level. However, although revealing the nature, the causes and the spectrum of genomic alterations in malignant cells are fundamental for understanding the transformation/transition states of the cells leading to acquisition of the malignant phenotype, final elucidation of the actual pathogenetic mechanisms responsible for conferring this characteristic phenotype upon cancer cells awaits complete delineation of the spectrum of proteome alterations and changes in malignant cells since all aspects of the malignant phenotype are determined and regulated by the proteome profile of the cell. This delineation of proteome alterations in malignant transformation, apart from its importance in clarifying many aspects of cancer biology and cancer genetics, is crucial for designing proper and effective genetic therapies for cancer based on approaches trying to manipulate abnormal proteome alterations in cancer cells by different mechanisms including augmentation of synthesis of normal instead of oncoproteins, suppression of and blocking the synthesis of oncoproteins, correction of structurally defective proteins, correction of trafficking defects of proteins and delivery of protein molecule capable of inducing selective inhibition/blockage/disintegration of key networks responsible for maintaining the malignant phenotype of cancer cells.

Understanding the malignant transformation of cells/development and progression of cancer would not be possible except after revealing the exact genomic components and the regulatory mechanisms responsible for, and underlying the,

growth of a single fertilized cell, the zygote, to a fully developed organism. Meticulous analysis of currently known facts/findings/observations related to the phenomenon of malignant transformation of normal cells to cancer cells adds more support to the hypothesis attributing carcinogenesis to defective temporal imprinting, not only of genes that regulate cell growth/proliferation and differentiation in early stages of development, but also of a wide spectrum of genes acting cooperatively in synchronization within a strictly defined framework comprising the three main constituents of the genetic material, viz. the genome/the transcriptome/the proteome, and aiming at preservation of the three main features of biological life, viz. genetic integrity/genetic stability/genetic identity. The behavior of malignant tumors is conceivable and clearly interpretable within the context of biological evolution as acquisition of a new phenotype with selective advantages over the current conventional phenotype, proliferation, spread, suppression of apoptosis, metastasis and formation of new tumors at multiple sites can be looked at as persistent trials to expand the size of the genome and to improve its survival potential, compared to mother cells. Paradoxically, in contrast to expected evolutionary improvements, the behavior of malignant cells results in deleterious effects on the organism leading to loss of integrity of the genome as observed in occurrence of marked hypodiploidy/hyperdiploidy of tumor cells, loss of genomic stability caused by, and observed as, small and large deletions/enhanced rates of chromosomal rearrangements/breaks and structural aberrations detected in most cancer cells during most of their life spans, as well as loss of genomic identity due to acquisition of new different distinctive phenotypes that bear little resemblance to those of the parent phenotypes and that get more divergent along their progressive course of spread and survival. There is no satisfactory explanation of this paradoxical fate of malignant cells characterized by final extinction in spite of the marked selective advantages they have over normal cells. Also, there is no indication of possible formulation of such an interpretation in the near, or even in the far, future. Malignant transformation, at the end, might prove to be the biological mechanism responsible for terminating life at the molecular level.

10. Clonal origin of tumors

There are two main concepts regarding the cellular origin of cancer. The hypothesis of the monoclonal origin of tumors proposes that most neoplasms arise from a single cell of origin, and tumor progression results from acquired genetic variability within the original clone allowing sequential selection of more aggressive sublines of the mother cell [15]. Within this context, carcinogenesis has long been considered as a progressive multistage pathophysiological process induced by pathogenetic mechanisms leading to malignant transformation of one single cell turning it into cancer cell and spreading to its daughter progeny resulting in formation of a tumor.

The opposing hypothesis that postulates the polyclonal origin of tumors, attributes tumor development to a situation where two or more cells or clones of cells interact to initiate a tumor. This postulation rests on a number of findings including numerous examples, encompassing 24 different types, of tumors with X-linked marker heterotypy, unequivocal demon-

strations of polyclonality in chimeric models of rodent and human tumors, mutational data consistent with polyclonal tumor origin where initiated cells are much more common in normal tissues than previously realized, and the observation that while tumors have higher levels of mutation than normal tissues, oncogenic mutations frequently are present as subpopulations within tumors, rather than as the pure mutant populations expected to develop from a single initiated cell [16].

As referred previously, it is hard to accept the postulation of tumor development from a single normal cell that gets transformed into a malignant cell by one or few mutations affecting one or more cancer-related genes of the genome. The malignant phenotype is not a result of just few mutations, rather it reflects a radical widespread change of the genome. The classical conventional definition of mutational events entails any structural changes of parts of the genome, ranging from change of a single base up to alteration of the whole genome, that occur either spontaneously or, more commonly, induced by exogenous environmental mutagens leading to deleterious functional consequences. This definition does not fit properly, or even approximately, to the types/nature/effects of mutations leading to malignant transformation. There are too many reasons for this conclusion. *First*, whereas most mutations result in deleterious functional consequences of affected cells including loss of cellular function(s), induction of apoptosis and accelerated degeneration and cell death, none of these detrimental effects are observed in cells undergoing malignant transformation. Instead, malignant transformation of normal cells to cancer cells results in acquisition by cancer cells of new functional and morphological phenotypes encompassing numerous selective advantages over normal cells. *Second*, the large scale extensive structural/functional genomic alterations of the magnitude seen in normal cells undergoing malignant transformation cannot be caused by, or solely attributed to, one or few mutations. *Third*, the obvious purposeful nature of malignant transformation of normal cells to cancer cells coincides with the conventional rules of biological evolution, taking into consideration the numerous selective advantages conferred upon malignant cells as regards metabolic competence, proliferative potential, regenerative abilities, survival span and many others. Although a paradoxical fate of cancer cells finally ensues leading to extinction rather than preservation/expansion/evolution of the genome, this happens because of devastating complications related to the whole organism, e.g., under nutrition/immunodeficiency/organ failure, and does not change the fact that malignant transformation represents a positive selective advantageous evolutionary stage of biological life of normal cells undergoing evolutionary transitions to cancer cells.

11. Genetic therapies of cancer

Malignant tumors have many challenging characteristics that make their effective treatment by currently available conventional approaches an exceedingly hard task to achieve. The reasons for this conspicuous failure of treatment of most tumors are many. *First*, tumors begin as tiny growths undetectable by current imaging techniques and, in most instances, causing no pathognomonic signs or symptoms except after attaining relatively sizable masses. *Second*, early metastasis of cancer cells, adds a tragic aspect to the story as metastatic tumors, which

constitute the major and commonest cause of death of patients with cancer, can migrate to multiple sites, grow in many organs and remain undetected until their overwhelming complications begin to cause clinical manifestations that allow for their late diagnosis. *Third*, in view of considerable lack of knowledge regarding the, still unrevealed, structural and functional aspects of malignant cells, no specific therapy capable of selectively targeting tumor cells exists. Current treatments aiming at inhibiting tumor growth, e.g., antiproliferative chemotherapeutic agents, or killing tumor cells by different means, e.g., apoptosis inducing agents and cytotoxic drugs, are not selective against cancer cells, they rather act indiscriminately on malignant as well as on normal cells, thus causing damage to both, sometimes even causing more damage to normal cells because malignant cells can construct novel metabolic pathways allowing them to adapt to the effects of these toxic agents. *Fourth*, the metabolic consequences of the malignant phenotype on host cells/tissues/organs are numerous and diverse making their treatment a real health burden on patients with cancer in view of the multiple approaches needed to combat these effects. For instance, a tumor can cause dysfunction of vital organs leading to many pathophysiological alterations, bone marrow depression leading to anemia/leukopenia/thrombocytopenia, immune deficiency resulting in recurrent infections, pressure effects on vital organs and many other consequences and life threatening complications that necessitate intolerable therapeutic intervention in most cases.

Current therapies for cancer comprise two main approaches: conventional therapies and genetic therapies. Conventional therapies of malignant tumors comprise three main approaches: *surgical* intervention aiming at radical excision/removal of early localized tumors or symptomatic debulking of large non-excisable tumors, *radiotherapy* aiming at damaging malignant tumors in view of their sensitivity to radiations in addition to the direct cytotoxic effects of radiation on living cells, *physical* approaches including cryotherapy or cryosurgery where killing of cancer cells is attained by freezing by liquid nitrogen or argon gas, thermal therapy or hyper-thermia treatment of cancer where high temperature (45 °C or 113 °F) is used to kill malignant cells, and laser therapy where laser lights of different kinds (carbon dioxide (CO₂) lasers, argon lasers, and neodymium:yttrium–aluminum-garnet (Nd:YAG) lasers) are used to kill tumor cells. Each of these conventional approaches for cancer treatment (surgical intervention, radiotherapy and physical techniques) has its indications and its contraindications, its advantages and disadvantages and its varied techniques. In view of the marked advances in trials/researches/applications aiming at improving the technical aspects of these conventional approaches, it is hoped that treatment and/or alleviation of a considerable sizable portion of many types of cancer would be achievable in the future.

Genetic therapies of cancer refer to treatment modalities designed for, and directed against, the three main constituents of the genetic material of the cell: the genome, the transcriptome and the proteome, and include innumerable modalities each aiming at targeting one or more aspects of the malignant phenotype. Examples of these treatment approaches include inhibition of cell growth and arrest of cell proliferation, induction of apoptosis via enhancement of apoptotic pathways, inhibition of formation of new vascular networks or angiogenesis which has a crucial role in maintaining tumor survival and metastasis, synthesis of antagonistic or interfering oligonucle-

otides that bind in a complementary manner to mRNAs transcribed by oncogenes leading to cessation of synthesis of oncoproteins, augmentation of the efficiency of the immune system in attacking and embracing malignant cells, functional nullification of harmful functions and lethal effects of oncoproteins by synthesized antibodies specific for particular oncoproteins, selective destruction of cancer cells by oncolytic virotherapy using genetically engineered viruses designed to infect cancer cells and induce cell death through the propagation of the virus and expression of cytotoxic proteins leading to tylosis of cancer cells, interference with cell growth and changing the intercellular microenvironment surrounding malignant cells by direct gene transfer approaches, blockage and inhibition of expression of cell surface oncoproteins that interfere with intercellular connections and cell adhesion molecules and pave the way for disintegration/migration/metastasis of cells, blockage and interference with signaling oncoproteins that have key roles in mediating crucial intracellular and intercellular functions responsible for maintaining and promoting the malignant phenotype, enhancing susceptibility of cancer cells to toxic effects of cytotoxic drugs and antiproliferative agents used to combat their growth, invention of large numbers of cancer vaccines or biological response modifiers designed to work by stimulating/restoring/augmenting the immune system's ability to fight cancer cells.

The list of therapeutic approaches to genetic therapies of cancer is endless in view of the functional versatility of tumor cells. One new emerging approach in this respect, viz. use of natural products extracted mostly from plants that have profound inhibitory effects on cancer growth and metastasis, however, is worthy of more consideration as it, probably, represents the least damaging and more safe treatment modality for cancer. Examples of these natural products include the small biologically active flavonoid genistein found in high amounts in soya [17], many plant hormones that can induce apoptosis of malignant cells in addition to other anticancer/antiviral/antiproliferative/antiangiogenic actions including sodium salicylate [5], Jasmonic acid and methyl jasmonate [6], Brassinosteroids [7], gibberellin derivatives [8], in addition to many natural products of plant origin including Vinca alkaloids/Taxanes (Paclitaxel and docetaxel)/flavopiridol/homoharringtonine/ β -lapachone/combretastatin A4, of microbial origin including rapamycin and geldanamycin which are macrolide compounds obtained from *Streptomyces hygroscopicus*, and of marine sources that have varied beneficial effects in inhibiting growth/proliferation/metastasis of malignant cells and enhancing their susceptibility to toxic effects of cytotoxic drugs through different mechanisms targeting oncoproteins/enzymes/signaling pathways/cytoskeleton alterations/etc., [18].

Effective genetic therapies of cancer have to fulfill particular requirements in view of the nature of the disease because of the numerous pathogenetic mechanisms underlying its initiation/persistence/progression and the widespread pathophysiological complications caused by the original tumor as well as by tumor metastases. In order to be approved for cancer therapy, the medications used must be selective in action targeting only, if possible, tumor cells and avoiding as much as possible normal cells. Complications caused by side effects of cancer medications must be tolerable and amenable to alleviation by other therapeutic approaches. Selective targeting of the crucial key features responsible for mediating the lethal effects of the malignant phenotype, particularly enhanced proliferation and

unopposed apoptosis, angiogenesis and metastasis to distant organs represents a pivotal goal of research work in this regard.

Unfortunately, current available knowledge about the different functional aspects of the malignant phenotype does not sufficiently allow for designing or synthesis of safe, selective and effective medications of cancer. However, there are few guidelines for research efforts in this respect that might be worthy of consideration.

First, exclusive gene therapy trials aiming at direct targeting of specific genes of the cancer cell genome are not expected to achieve any success, on the contrary, they represent the worst approaches because of the equal or more damage they cause to normal cells as well, because of their haphazardous uncontrollable non-selective action(s). Currently defined framework of cancer biology and the nature of malignant transformation exclude direct targeting of cancer-related genes from being an effective treatment approach to cancer.

Second, the theoretical impetus of most immunotherapeutic approaches of cancer treatment is based on the postulation that tumors develop as a result of defective/deficient functioning of the immune system. Though this assumption is partially correct, it cannot be relied upon for designing or formulating efficient cancer immunotherapies. Currently available immunotherapies for cancer comprise different modalities of cell-based therapies/antibody therapies/cytokine therapies and other less common approaches. Most of these modalities depend on specific targeting of particular oncoproteins involved in initiation/progression of tumors. In spite of the crucial role played by the immune system in defense processes and preservation of many aspects of biological homeostasis, its presumed goals in efficient treatment of cancer seem hard to accomplish. Successful treatment of cancer by immunotherapeutic agents is limited, practically, to hematological malignancies where separate cells can be targeted individually. Though targeting of outer border cells of solid tumors can be achieved by monoclonal antibodies directed selectively against surface oncoproteins, they have limited, or no, effectiveness beyond this stage when tumors attain sizes and form growths that can neither be embraced nor penetrated by immunotherapeutic agents. The efficient capabilities of malignant cells to adapt to counteracting immune factors by different means, e.g., synthesis of oncoproteins that kill immune cells or nullify their detective abilities to detect self from non-self components, constitute an additional hindrance to development of reliable and/or effective immunotherapies of cancer.

Third, the use of naturally occurring products extracted from plant/microbial/marine sources in treatment of malignant tumors seems quite promising in view of its apparent safety and effectiveness, with least harm being induced to normal cells or to managed patients. However, the need for higher pharmacological doses of these agents to work efficiently and the possibility of causing side effects due to mixing with other extracts of the same source necessitates more innovative techniques for purification to attain maximal anticancer effects and maximal clinical safety for managed patients.

Fourth, irrespective of theoretical postulations upon which different genetic therapeutic approaches to cancer are formulated and designed, final success in achieving efficient treatment of cancer depends wholly on accurate comprehensive delineation of the oncoprotein profile of malignant cells and on proper understanding and interpretation of the mechanisms

mediating and executing the functional properties and the morphological features of the malignant phenotype. As in normal cells, where the normal proteome of the cell is responsible for conducting all cellular functions, the proteome of malignant cells, or the oncoproteome, mediates all life activities of cancer cells including basic essential features like growth/proliferation/metastasis as well as all subsidiary activities needed to maintain the malignant phenotype, to consolidate its persistence as well as to augment tumor spread and metastasis.

Fifth, genetic therapeutic approaches to combat cancer could involve varying treatment modalities targeting any or all of the genetic constituents of the cancer cell, viz. the genome, the transcriptome and the proteome. Therapies targeting the genome are meaningless waste of resources/efforts/time in view of lack of sufficient knowledge of different aspects of genomic alterations of malignant cells. Targeting the transcriptome and the intimately related microRNA components, that have conspicuous pivotal regulatory roles exerted over most functional genomic regions, is a reasonable approach being more safer than non-selective random targeting of nuclear genes. The relative accessibility of the transcriptome, mRNAs, in the cytoplasm by oligonucleotides is a favorable advantage for this treatment approach. However, selective targeting of specific strand segments is a prerequisite for successful effects, otherwise, complementation with other transcribing segments leading to inhibition of translation and synthesis of other useful proteins might result. Similarly, interference with regulatory microRNA components needed to maintain the expression of genes that hinder the progression of malignant transformation, e.g., through suppression of overexpression of oncogenes, would result in dreadful consequences leading to paradoxical enhancement of tumor progression and spread.

Sixth, therapeutic approaches aiming at corrective manipulation of the oncoproteome rather than the genome, the transcriptome or the regulatory microRNA system, represent the most logical and feasible approaches toward designing radical effective genetic therapies of cancer as they comprise direct targeting of factors that determine the functional and structural aspects of the malignant phenotype. Focusing ongoing researches in this direction would, probably, result in formulating more fruitful treatment modalities for cancer.

12. Conclusions

Malignant transformation of normal cells to cancer cells represents an enigmatic phenomenon because of the many ambiguous controversies embodied within most of its aspects. Cancer is a peculiar biosystem that has its own rules that dictate and regulate all the structural features/functional properties/interactive potentials of its components. Unfortunately, the majority of these rules are, still, unknown. More importantly, in view of acquisition of numerous selective advantages due to the wide spectrum of variations/capabilities/resilience of the proteome of malignant cells, or the oncoproteome, these rules defined by the malignant phenotype dominate over conventional biological rules regulating life aspects of normal cells. Though understanding the genetic implications of this dominance, including the evolutionary expansion and progression followed by extinction of the genome of the malignant cell, or the oncogenome, and the impaired decay or apoptosis of transformed cells, is critical

for interpreting the developmental origin and behavior of tumors, it also comprises an appreciable achievement in revealing the dynamics of cancer cells which is pivotal in designing and formulating effective treatment modalities against development/progression/metastasis of cancer.

Although the exact nature of the basic cause of development of cancer is quite vague, current hypotheses regarding the pathogenetic mechanisms that possibly underlie development of malignant tumors comprise lots of speculations including defective temporal imprinting of the vast majority of developmental genes leading to mass reactivation/suppression of the genome with regaining the characteristic genetic profiles of early embryogenesis and development and spontaneous or mutation-induced functional imbalances between proto-oncogenes/oncogenes/tumor suppressor genes leading to abnormal/defective/altered regulation of cell division and growth. Defective parental imprinting might also be implicated in pathogenesis and development of hereditary tumors.

Irrespective of all suggestions, the development of the malignant phenotype of cancer cell reflects a radical change of the cell genome. This alteration involves extensive reprogramming/deprogramming of sizable portions of the genome with consequent reforming of the regions of active/silenced genes, quantitative transcriptome and micro RNAs components and proteome profiles. The newly reformed/regained oncoproteome reforms the cell architecture involving new features, some of which are identical to those of embryonic and fetal cells, and mediates all the functional capabilities of the malignant cell, thus conferring upon the cell the structural features and the functional properties of the malignant phenotype.

Metastasis represents the major cause of lethality of cancer in view of the progressive and relentless detrimental widespread changes of the structural components and the functional capabilities of tissues and organs affected by metastases. Though the exact nature of the cause of this mysterious behavior of malignant tumors is still obscure, many mechanisms responsible for mediating and regulating the dynamics of this process have been revealed including changes of cell cytoskeleton and cell movement/cell interactions/new angiogenesis and modulation of the extra cellular microenvironment. The hypothesis referred to in this article speculating a similarity of mechanisms regulating tumor cell metastasis and quorum sensing pathways of bacteria might be worthy of consideration since detection of postulated regulatory signaling pathways that have a role in tumor metastasis might pave the way toward designing and formulating effective treatment for this drastic process responsible for lethality of malignant tumors.

Within a clinical context, cancer, with very few exceptions, is a dreadful disease that ends lethally, whereas within a biological context cancer is a peculiar biosystem with its own rules that are largely unknown. The current disappointing situation as regards research trials aiming at constructing effective treatments for cancer might be attributed, in part, to incomplete recognition of the significant differences between these two contexts of malignant transformation. Although the peculiar characteristics of cancer as a self-dependent biosystem are well studied and well defined, the basic dilemma of malignant transformation continues to exist: we know, largely, how things happen but we do not know, to any extent, why they happen.

In spite of the innumerable treatment modalities designed/constructed/formulated and tried for treatment of most types of malignant tumors, effective cure of cancer is still very far from being achieved, desperately even a practical impossibility. Successful therapy of cancer is dependent on comprehensive understanding of the true nature of the genetic alterations causing malignant transformation of a normal cell to cancer cell and disclosure of the underlying mechanisms regulating growth/progression/metastasis and survival of malignant cells.

Away from palliative surgical excision/removal/debulking of tumors, current treatment approaches target one of the three components of the genetic system of the cell: the genome, the transcriptome and the proteome. Though the logic that motivates researches aiming at formulating genome-targeting therapies for cancer is quite reasonable, as cancer is primarily a genetic alteration, lack of essential basic knowledge regarding the different aspects of this alteration, in addition to lack of exclusive selective targeting of genes/segments/regions responsible for malignant transformation adjourn successful formulation of such effective genome-targeting therapies. Transcriptome-targeting therapies and regulatory small/microRNAs-targeting therapies of cancer are promising approaches in this respect, however, they are hampered by similar lack of sufficient basic knowledge regarding the extremely wide functional spectrum of their different components and lack of exclusive selective targeting of these components. Till comprehensive disclosure of the causes of carcinogenesis is attained, treatments of cancer based on different strategic concepts, viz. proteomic therapies rather than genomic therapies, might, hopefully, be the best approaches available in the fight against cancer in the current as well as in the coming era.

Conflict of interest

The author declares no conflict of interest.

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Additional resources

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