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REVIEW

Cancer: evolutionary, genetic and epigenetic aspects

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Abstract There exist two paradigms about the nature of cancer. According to the generally accepted one, cancer is a by-product of design limitations of a multi-cellular organism (Greaves, Nat Rev Cancer 7:213–221, 2007). The essence of the second resides in the question "Does cancer kill the individual and save the species?" (Sommer, Hum Mutat 3:166–169, 1994). Recent data on genetic and epigenetic mechanisms of cell transformation summarized in this review support the latter point of view, namely that carcinogenesis is an evolutionary conserved phenomenon—*a programmed death of an organism*. It is assumed that cancer possesses an important function of altruistic nature: as a mediator of negative selection, it serves to preserve integrity of species gene pool and to mediate its evolutionary adjustment. Cancer fulfills its task due apparently to specific killer function, understanding mechanism of which may suggest new therapeutic strategy.

Keywords Carcinogenesis · Mutagenesis · Epigenetics · Programmed death of organism · Evolutionary origin of cancer

Introduction

In scientific progress, there are periods of revolutionary change of paradigms, i.e. of certain basic ideas (Kuhn 1970). This is what is probably taking place today in our understanding of cancer. Strange as it may be, the huge interest in the scientific community to the mechanisms of carcinogenesis goes in parallel with the virtual lack of interest to some basic questions, such as "What is cancer? What is the nature of this phenomenon?" In only very few studies, these questions do become an issue of discussion.

The current paradigm holds that cancer is a consequence of internal imperfections of a multi-cellular organism, an evidence of failure of its adaptation mechanisms: the Darwinian evolution does not expect the future and does not plan for it, it is compelled to operate with means that are only available for it at present, "at hand", which makes compromises and restrictions inevitable (Greaves 2007). Cancer is thereby put into the same category as other illnesses of the old age. It is strange, but the notion of cancer as a consequence of imperfection of the organism, not having in fact serious justifications, has become something self-evident.

At the same time, cancer likening to other diseases seems arguable as it ignores one cardinal distinction. At the heart of a "usual" illness there is a decrease in function of the corresponding organ (irrespective of its causes). For instance, cardiovascular diseases arise because of decrease in the contractile functions of the heart and/or the conducting ability of the vascular network; diabetes, due to insufficient production of insulin; and Alzheimer's disease, due to infringement in nerve conduction, etc. The general pathogenesis of these illnesses is clear, even if there are no data on concrete molecular mechanisms.

Cancer is a different case as it involves appearance of a previously non-existent structure (tumor), realizing a previously non-existent function (destruction of the organism) and rendering powerful and versatile influence on the body. It is a question, thus, of not about a loss of an old function, as in "usual" illnesses of the old age, but rather about gain of a new function. Despite one-and-

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a-half centuries of persistent research, there is no clear understanding what this phenomenon is, neither is there an effective protection against it or means of combating it.

This contradiction has generated a number of new explanations of this phenomenon (Graham 1992; Kozlov 1996; Leroi et al. 2003). The new paradigm is most clearly expressed in the article by Sommer (1994), the very title of which "Does cancer kill the individual and save the species?" conveys in the implicit form its basic ideas: (1) cancer carries out a certain function and hence is not a by-product but a direct result of evolution; (2) the cancer's role is dual—being pernicious for the individual, it is necessary for the species; (3) cancer is an altruistic phenomenon, as price for the rescue of the species is self-destruction of the individual. A conclusion suggests itself that the ability of the cancer cell to kill the individual (its so-called killer function) (Lichtenstein 2005b) is the key property, which has determined the very appearance of this phenomenon.

The new understanding of cancer distinguishes oncology from a number of other medical disciplines. Their purpose is to struggle against errors of nature and restore the normalcy (this is what the doctor does when he eliminates a defect of a mitral valve, inserts an artificial teeth or prescribes a hypotensive drug). Oncology, on the contrary, is probably a unique medical discipline, which combats not deviations from norm and mistakes of the nature, but rather nature as such (because it struggles with a natural biological phenomenon). In this respect, oncology is somewhat similar to gerontology, which also struggles with a natural phenomenon of ageing. It is perhaps for this reason that in the both cases the struggle is so tremendously difficult.

In the recent years, new knowledge about carcinogenesis has accumulated. The whole body of this knowledge allows, apparently, replacing the interrogative sign in the name of Sommer's article with the affirmative one. In this review, different aspects of carcinogenesis are considered from a single point of view, namely, that carcinogenesis is an evolutionary conserved phenomenon (a programmed death of an organism) and serves to maintain the integrity of the species gene pool. With this in mind, I consider in the beginning various aspects of evolutionary origin of cancer, such as evolutionary conservation, the Peto paradox, regularity of this phenomenon ("The evolutionary role of cancer"). In "Cancer as programmed death of organism", the concept of cancer as programmed death of an organism is further substantiated by showing it as the process embracing the whole organism and unfolding along predetermined pathways. It is characterized by a regular and predictable change of stages with a striking coordination of molecular, cellular and tissue processes within the tumor/body system. The very important moment is that the killer function, this necessary attribute of a malignant tumor regardless of its type, origin and localization, cannot be explained from the accepted paradigm but fits naturally into the alternative one. The molecular mechanisms underlying this suicidal program are considered in "The genetics and epigenetics of carcinogenesis".

The evolutionary role of cancer

The evolutionary conservation of a biological phenomenon is one of the most powerful evidences of its importance: the stronger nature keeps its possession, the higher is its value. Evolutionary conservation of cancer is amazing: tumors are found not only in a multitude of now living animals (from molluscs to mammals), but also in fossil remains of dinosaurs of the Jurassic period (Greaves 2007; Leroi et al. 2003). These facts alone certify for the evolutionary value of this phenomenon.

Peto paradox and the transformation resistance of cells

The ability of cancer to affect various species of animals is a paradoxical fact. The matter is that carcinogenesis is closely related to cellular proliferation: due to cell divisions, accumulation of mutations in the cellular genome is possible (see below). Though the μ value (mutation rate per gene and cell division) varies greatly (Vijg 2000), its existence and non-zero values are evidence of this intimate link. Proliferation and transformation are thus conjugate processes: if proliferation is sufficiently intensive, the appearance of a completely transformed (i.e. cancer) cell is a matter of time.

This logic suggests that the probability of cancer increases with the mass of the animal (as the number of proliferating cells and the probability of mutations in them are higher) and its life span. It may be assumed, therefore, that if the resistances of animal cells to malignant transformation were the same, blue whales (weighing more than 100 tons) would all be suffering from cancer and disappearing as a species, whereas mice (weighting about 20 g) would not have cancer at all. In fact, however, nothing of this kind takes place: all species (whales, mice, and man) are susceptible to cancer but neither becomes extinct.

Explaining this so-called Peto paradox (Peto et al. 1975) is possible by assuming an inequality of transformation resistance of cells of various species (in whales it should be much higher than in mice). One of the most important determinants of the transformation resistance is the number of stages of transformation: the greater this number, the more strongly the individual is protected against cancer (Frank 2004). Indeed, fibroblasts of man are much more difficult to transform than fibroblasts of mouse because of a greater number of mutations required for this purpose in the former (Hahn and Weinberg 2002). Thus, the Peto paradox (the more or less equal susceptibility to cancer of animals

strikingly differing in mass and life span) is the evidence of species-specific adaptation of cell transformation resistance. If cancer had any unfavorable influence on viability of a species, then its ousting beyond the limits of the species' life span by means of augmentation of the transformation resistance would be easily achieved. In one peculiar case of the naked mole rat, in which tumors have never been observed, this extraordinary resistance to cancer has come about due to a relatively slight modification of regulatory network, namely, the involvement of both p16(ink4a) and p27(Kip1) tumor suppressors in the control of contact cell inhibition (in human and mouse, as opposed to mole rat, contact inhibition is triggered only by the induction of p27) (Seluanov et al. 2009). The reason for the appearance of such a unique trait remains unknown, but this exception to the general rule shows that elimination of cancer, if necessary, would present no difficulty. The susceptibility to cancer of most animals, by the rule of contraries, is therefore the evidence of its evolutionary usefulness.

Malignant transformation affects different species and different tissues. The latter, too, differ in their mass and proliferation rate and, accordingly, in transformation resistance: in fact, for tumor appearance in different human tissues, different numbers of mutations are required (Renan 1993). This is because cell type-specific differences exist in the requirements for tumorigenic transformation (Rangarajan et al. 2004). The fact that transformation resistance of cells is not only species-specific but also cell type-specific indicates that genetic as well as epigenetic mechanisms participate in its formation.

Cancer: randomness or regularity?

The probability of appearance of a tumor in an individual is a function of intensity of mutagenesis, which in turn is determined by the balance of two opposite processes: mutagenesis and anti-mutagenesis. Mutagenic factors are subdivided, by their origin, into two types: exogenous and endogenous. Factors from the external environment are chemical carcinogens, ultraviolet irradiation, ionizing radiation, viral and bacterial infections. This "external" component of mutagenesis is subject to certain correction and is therefore an important object of preventive actions.

The "intrinsic" component of mutagenesis, that cannot be controlled at present, is caused by fundamental processes: cell division entails inevitable errors of replication; metabolic processes and respiration lead to accumulation of aggressive reactive oxygen species (ROS); telomere shortening during cell divisions results in chromosome aberrations; spontaneous DNA depurination and methylcytosine deamination bring about DNA damage; phagocytosis of apoptotic bodies is related to a wide-scale natural transfection.

Mutagenesis is opposed by mechanisms of DNA repair and apoptosis that are capable of strongly, by many orders of magnitude, reducing mutation rate but incapable of eliminating it completely. As a result, vital activity is accompanied by a parallel mutagenesis and, as its consequence, the organism is gradually "creeping up into carcinogenesis" (Lichtenstein 2009). The speed of this process, which determines the probability of appearance of a tumor in the individual, is, as a rule, low. Thanks to that, the majority of people throughout their life do not form tumors (according to a saying, "not everyone lives long enough to reach his/her cancer"). However, in about 20% of people, the balance of counteracting forces (external and internal mutagenesis, on the one hand, and protective mechanisms of DNA repair and apoptosis, on the other) develops unfavorably, the process of "creeping into carcinogenesis" is accelerated and, as a result, cancer overtakes the individual in, depending on the acceleration rate, elderly or even young age (in the latter case, hereditary genetic defects often play a key role).

Thus, the prevalent opinion on randomness of cancer, based, first, on a relative rarity of the disease (overall, two out of ten people suffer) and, second, on the fact that cancer is invoked by random events (mutations), requires apparently a certain revision. Cancer is most likely a natural, but not inevitable, phenomenon. The same can be said about old age: after all it is also natural but not inevitable (it is quite possible to die young).

A completely transformed cell arises after several rate-limiting events (mutations) affecting a number of key genes (oncogenes and suppressor genes). The number of such driver mutations responsible for driving the initiation, progression, or maintenance of the tumor varies, depending on cell origin, from 4 to 12–15 (Renan 1993; Wood et al. 2007). Mutations are rare events, and accumulation of such a large number in one cell is hardly probable. If it nonetheless occurs, this happens because with every cancer-promoting mutation the cell gets some selective advantage and forms a clone. What is improbable for a cell is quite probable for a clone, and the bigger the clone, the higher is the probability of the next mutation in one of the daughter cells. The process then repeats itself. Thus, according to the theory of multi-stage carcinogenesis, cell transformation is a Darwinian process of consecutive cycles of mutation–selection (Armitage and Doll 1954).

Accumulation of the mutant cells in a population is symbolized by "the mutational pyramid", at which base are cells with the lowest number of cancer-promoting mutations, and at the top, with the greatest (Lichtenstein 2005b). As mutagenesis is progressing (and the mutational pyramid grows in width and height), the transformation resistance of the cell is depleted, thus serving as a countdown trigger. Its full depletion in the cell, which is at the top of the pyramid

(i.e. completely transformed) marks the transition of carcinogenesis from the latent phase (in which the "underground" of the tumor, consisting of precancerous cells at different stages of transformation, is formed) to an open clinical phase (when tumor becomes evident) (Lichtenstein 2006).

An obligatory presence in each tumor of an "underground", the assumption that follows from general reasoning, is supported by discovery of "field cancerization", i.e. large and surrounding the tumor "patches" of partially transformed cells, recognized on the basis of mutations in *TP53* but remaining undetectable by routine diagnostic techniques (Braakhuis et al. 2003). The impossibility of defining the borders of these fields and completely removing them during surgical operation is, apparently, the principal cause of tumor relapse.

Mutagenesis takes place in all cells of the body, and, hence, in each tissue a mutational pyramid grows. Their growth can be compared to sprinting on parallel lanes: all runners are running, but the winner (in this case, who first generates a tumor) is determined by casual circumstances. If the speed of the leader drops for some reason, its place is taken by another runner. This explains the so called "effect of communicating vessels" (as it was named by a Russian physician J.I. Lorie): a decrease in incidence of one form of cancer often does not result in a decrease in overall cancer incidence—the "niche" is filled with other forms (for instance, a drop in stomach cancer among the Japanese who moved to the US, is compensated by a rise in the incidence of lung cancer). Thus, one can conclude that tumor localization is random, but its appearance is natural (owing to the constantly going and progressing mutagenesis). The fact that not every individual faces an oncologic disease during life is explained, apparently, by a prevailing mortality from other causes.

Cancer as a guardian of gene pool

The recently proposed concept of phenoptosis (programmed death of an organism) postulates an existence in biology of the so-called Samurai principle ("it is better to die than to be wrong"). The biological system is suggested to possess a built-in self-destruction program. This program is not active usually and comes into effect when the system begins to pose hazard to another one, which is standing above in the biological hierarchy (Longo et al. 2005; Skulachev 1999). The most fundamental manifestation of this altruistic principle is apoptosis, which eliminates a cell when it begins to be a threat for the multi-cellular community. At a signal from the outside, the cell makes a suicide for the sake of maintenance of the tissue homeostasis and renewal, or as a necessary condition of morphogenesis during embryonic development. The signal, which activates the self-destructive program, can arise also inside the cell (e.g. in the case of DNA damage).

If cancer is also a case of phenoptosis, a question arises, "Which advantages does it confer to the higher hierarchical system—to the species?" Apparently, these are the same that apoptosis confers to the multi-cellular community, namely, the negative selection of the hazardous individuals. Indeed, in hereditary cancer syndromes (when the "sanitary" role of cancer most evidently manifests itself), the tumors appear in reproductive age and are often multiple, what does not leave the individual any chance of survival. By this means, germ-line mutations of functionally important genes, which otherwise would have a high probability of spreading in the population (Lichtenstein 2005b, Lichtenstein 2008), are generally eliminated from the gene pool (Ponder 2001). A computational model of cancer progression suggests that an additional protection against cancer would lead to significantly increased genetic predisposition to disease in the population as a whole (Frank 2004).

The fact that cancer mainly affects individuals of old age (>95% of total incidence) is often considered as an argument that it is selectively neutral. Such an interpretation raises doubts. A functionally important program, which is active at the reproductive age, will most likely be also active at an old age (even if it becomes useless or even harmful), since evolutionary mechanisms that could correct a situation, are inefficient at that age (the theory of antagonistic pleiotropy) (Kirkwood and Austad 2000). Thus, the high cancer incidence at advanced age can be considered as a side effect of a program designed for elimination of young carriers of mutant genes (the number of such individuals is relatively low but they are potentially quite hazardous). Cancer-related genes belong to "essential genes" (classified as lethal by mouse knockout experiments), and their mutant forms are, probably, more detrimental to fitness than those in other genes (Thomas et al. 2003).

Another hypothesis calling into question the general assumption that cancer represents simply a breakdown in normal physiology, suggests that it carries out an important function of maintenance of optimal level of germ-line mutagenesis. A very low mutagenesis would not provide the variability necessary for evolution of species, while a very high mutagenesis is fraught with many unfavorable consequences, since for each advantageous mutation, there are many detrimental ones. In this case, cancer is a mechanism for negative selection, which limits the upper level of mutagenesis and ensures an optimal mutation rate (Sommer 1994).

And, finally, cancer perhaps fulfills the important function of quality control at times of fast evolutionary changes of the species, especially associated with a body mass or life span increase, i.e. when the genome is yet insufficiently adapted to the new conditions (such situation arises, for example, in selective breeding of large dogs,

which have, as a result, an extremely high cancer incidence) (Graham 1992; Leroi et al. 2003).

Cancer as programmed death of organism

In spite of the numerous variations of the oncologic process, its successive stages are so regular and the overall pattern is so invariably the same that one can foresee from the very beginning what will follow: growing tumor aggression, inefficiency of host defense, resistance to chemotherapy, emaciation of vital forces of the body, metastases and death. At any level of cancer research (clinical, cellular, or molecular), what comes first to one's attention is the programmed character of the unfolding events. From theoretical point of view, cancer may be viewed as an intrinsic robust state of the endogenous molecular–cellular network shaped by evolution that forms a nonlinear stochastic dynamical system with many stable attractors in its functional landscape. The genesis and progression of cancer are stochastic transitions between different attractors (Ao et al. 2008).

Where lies the blame for cancer: in the cell or organism?

There are no doubts that the tumor begins from one transformed cell: the monoclonal origin of a tumor is one of fundamental notions of oncology. In a model system, using a method for reconstructing cell lineage trees from genomic variability caused by somatic mutations, it was found that the tumor initiated from a single founder cell (Frumkin et al. 2008). Does it mean that this first cell is to blame for the disease and that the disease is a casual event? Or, in other words, that carcinogenesis is a cell-autonomous process? Most likely, the real situation is more complex.

An unexpected result of recent studies suggests that transformation of the epithelial cells, which give rise to a malignant tumor, is a secondary event, while the primary changes arise in the tumor environment (Maffini et al. 2004). A rat mammary tissue recombination model and the chemical carcinogen *N*-nitrosomethyl urea (NMU) were used to determine whether the primary target of the carcinogen is the epithelium, the stroma or both tissue compartments. Mammary epithelial cells were exposed in vitro either to the carcinogen or vehicle before being transplanted into the cleared fat pads of rats exposed to carcinogen or vehicle. Neoplastic transformation of these mammary epithelial cells was shown to occur only when the stroma was exposed in vivo to NMU, regardless of whether the epithelial cells were exposed to the carcinogen. Mammary epithelial cells exposed in vitro to the carcinogen formed phenotypically normal ducts when injected into a non-treated stroma. It is not known how universal is such a sequence of events, but is doubtless that a tumor and its environment participate in carcinogenesis as equal partners.

Throughout the long process of transformation, the cell resides in an environment of normal neighbors and is exposed to their influence, which could be pro- and anti-carcinogenic. Since Virchow's time, the association of cancer with a chronic inflammation is well known: not only does it always accompany tumor growth, but also very often precedes it (Balkwill and Mantovani 2001; Coussens and Werb 2002). Inflammatory cells secrete cytokines and chemokines, which bind to receptors on cancer cells and enhance their proliferation, migration and invasion.

There is evidence that carcinoma-associated fibroblasts (CAFs), which phenotypically differ from normal fibroblasts, possess the ability to stimulate carcinogenesis (Bhowmick et al. 2004; Olumi et al. 1999). The loss of TGF-β responsiveness in fibroblasts resulted in intraepithelial neoplasia in mouse prostate and invasive squamous cell carcinoma of the forestomach (Bhowmick et al. 2004). A question that remains unanswered concerns the so-called interdependent coevolution of clonal populations of carcinoma cells and CAFs, or, in other words, whether simultaneous generation of two symbiotic malignancies is possible. There are opposite opinions: some authors have found genetic alterations in CAFs, whereas others consider such findings as artifacts and deny their real existence (Allinen et al. 2004; Campbell et al. 2009; Eng et al. 2009; Weinberg 2008). No matter what the answer would be, one thing is clear: there are significant distinctions between normal and cancer-associated fibroblasts, these distinctions are heritable and are caused by specific patterns of gene expression (Allinen et al. 2004).

Senescent cells can also induce their normal neighbors to carcinogenesis. The state of senescence arises from the genotoxic stress of cells or depletion of their proliferative potential. Senescent cells secrete myriad of factors associated with inflammation and malignancy and acting by a paracrine mechanism. Premalignant mammary epithelial cells exposed to senescent human fibroblasts in mice undergo full malignant transformation. Obviously, senescent cells contribute to age-related pathology, including cancer (Coppe et al. 2008; Parrinello et al. 2005).

And, finally, conditions can exist in the body that favor predominant survival of the mutant cells with the altered metabolism. For example, under low-glucose conditions, mutations in oncogene K-*RAS* allow colorectal cells to outgrow their neighboring healthy cells with wild-type K-*RAS*. The cells with mutant K-*RAS* exhibit enhanced glucose uptake and glycolysis due to increased GLUT1 (glucose transporter-1) expression (Yun et al. 2009).

The capabilities of the environment are not limited to procarcinogenic effects. Tumor environment also possesses an opposite ability, namely to reverse tumor phenotype (this

could hardly be imagined if one takes into consideration the irreversibility of tumor-inducing mutations). Mintz and Illmensee (1975) found that a mouse embryonic blastocyst microenvironment suppressed the tumorigenic phenotype of teratocarcinoma cells, which could give rise to normal tissues. These experiments have refuted the established notion about irreversibility of tumor phenotype. Recent studies using various embryonic models confirmed the earlier data, and have shown, in particular, a possibility of reversion of the metastatic phenotype of aggressive melanoma cells. Nodal signaling was found to be especially important, the inhibition of which promotes the reversion of melanoma cells towards a melanocytic phenotype (Hendrix et al. 2007). Some signal pathways have been identified (in particular, the SIAH1, presenilin 1, TSAP6, and translationally controlled tumor protein, TCTP), whose activation in tumor cells can result in the negation of genetic defects and lead to tumor reversion. This process involves a reprogramming mechanism using epigenetic and probably genetic tools (Telerman and Amson 2009).

It can be concluded that both cell-autonomous events (genetic perturbations) and actions of environment may contribute to carcinogenesis. The former are random, while the latter are, most likely, non-random and depend on the genetic constitution of the organism, that is, on a multitude of factors, some of which may have no explicit relation to carcinogenesis (e.g. genetic predisposition to inflammatory reactions, senescence-associated secretory phenotype, etc.).

One can envision the internal organization of the cell and organism as complex hierarchy of densely interlaced molecular networks with an abundance of feedback loops. The tumor is so strongly integrated into these networks (Albini and Sporn 2007; Allinen et al. 2004; Balkwill 2004; Bissell and Radisky 2001; Gallagher et al. 2005; Hill et al. 2005), that it is impossible to "isolate" and consider it with no connections with its environment, exactly as in the case of any other tissue. Cooperation between tumor and its environment is well established, what allows to consider a tumor like a special "organ" (Bissell and Radisky 2001). Indeed, a tumor meets the formal definition of an organ as an anatomically discrete complex of tissues, integrated to perform specific functions and has the necessary attributes: stem cells (cancer stem cells [CSC]) (Reya et al. 2001), hierarchical structure often simulating a normal tissue constitution (Perez-Losada and Balmain 2003), cellular specialization (Axelrod et al. 2006), structural and functional unity with the microenvironment (Albini and Sporn 2007; Merlo et al. 2006), and, as has been noted above, a specific function – the organism death (Lichtenstein 2005a). Tumors produce both stimulators and inhibitors of angiogenesis in their microenvironment and exhibit a growth slowdown with a possible asymptotic approach to a final tumor size, or "set point", what indicates that the tumor maintains a vestige of normal tissue mass control. Developing tumors and organs may share an awareness of total mass through the exertion of an increasingly inhibitory influence on their own growths by way of increased natural angiogenic suppression. The difference is that the "set point" has been inappropriately set forward, allowing the tumor to kill the host well before the new set point is achieved (Hahnfeldt et al. 1999).

While the tumor/host relationships were earlier thought to be antagonistic (perhaps by analogy with infections), today their synergy (paradoxical "love, not war") becomes apparent (Lichtenstein 2008). In its destructive efforts, the tumor relies on the support of normal tissues, both the immediately surrounding (Albini and Sporn 2007; Bissell and Radisky 2001; Dranoff 2004) and remote (Dolloff et al. 2007; Hayward et al. 2001; Hiratsuka et al. 2006; Kaplan et al. 2005; Karnoub et al. 2007; Nolan et al. 2007; Sawyers 2007; Williams et al. 2007; Wyckoff et al. 2007). The participants of the "rescue operation" include fibroblasts (Elenbaas and Weinberg 2001; Kalluri and Zeisberg 2006; Orimo et al. 2005), tumor-associated macrophages (Condeelis and Pollard 2006; Pollard 2004; Sahai 2007), tumor-infiltrating neutrophils (Ardi et al. 2007), stroma (Albini and Sporn 2007), bone marrow (Nolan et al. 2007), remote organs (Hiratsuka et al. 2006). Owing to the help from the outside, the tumor gets blood supply, grows, has an increased mutation rate (Gupta and Massague 2006; Sahai 2007). In cell invasion experiments, it was found that stroma fibroblasts can lay tracks in the intercellular matrix and serve as leaders for epithelial cells that followed them (Gaggioli et al. 2007). Using animal models of breast cancer metastasis, it was shown that there exist subpopulations of macrophages that provide for tumor cell extravasation, survival and subsequent growth of metastatic cells (Qian et al. 2009). Bone marrow cells exposed to cytokines secreted by the tumor migrate to target organs and prepare the niche for future metastases (Kaplan et al. 2005). The enzyme lysyl oxidase secreted by hypoxic tumor cells was found to modify the extracellular matrix and also contribute to pre-metastatic niche formation (Erler et al. 2009). These findings are supported by gene expression profiling of both epithelia and stroma at specific time points during tumor progression, which reveal sequential enrichment of genes mediating discrete biologic functions in each tissue compartment (Reuter et al. 2009).

The complex interactions between normal and tumor cells result in formation of numerous "vicious circles" that drive the tumor progression. Though microenvironment sometimes reveals "yin-yang" activities, i.e. can be, depending on circumstances, either pro- or anti-carcinogenic (Wang et al. 2004; Witz 2008), the net balance steadily develops in favor of the growing tumor.

The role in carcinogenesis of the immune system, the main defender of the organism from all invasions, is dual. At the initial stages, the adaptive immunity maintains occult cancer in an equilibrium state (Koebel et al. 2007). However, after the clone escape and tumor formation the immune system begins paradoxically to stimulate its development (Balkwill and Mantovani 2001; de Visser et al. 2006; Prehn 1994).

Thus, the oncologic process appears as program of systemic disintegration with a role of the tumor as its organizer, coordinator and pacemaker.

Why does the cancer patient die?

The question of how and why a tumor kills the organism, owing to its importance, should apparently take the central place in cancer research: its solution could help, on the one hand, to get at the root of this disease, and on the other, to find effective treatment. Strangely enough, this question does not attract the attention it deserves, and a host of questions remain. Why the death of the cancer patient is inevitable? Whether is it a side effect of tumor growth, a result of exclusively local influence on normal tissues? Or, on the contrary, is the death predetermined and caused by a certain special activity of the cancer cell, is "beneficial" and for that very reason so evolutionary conservative?

The point of view that the tumor kills by the fact of its existence seems to be self-evident (metastases, in particular, are considered to be an exhaustive explanation of the fatal outcome). This viewpoint is based probably on the fact that in vitro cultured cancer cells do not exert a negative effect on their normal neighbors, do not produce toxic products and, most likely, are not capable of doing them in vivo. At the same time, their ability to spread and form metastases is obvious. It seems natural that it is the local effects on surrounding normal tissues that cause death of the body. In some particular cases, this is what happens indeed: local effects prevail, for example, in cases of profuse bleedings, brain compression, intestine perforation or obturation, etc.

However, malignant tumor evidently exerts also a general influence on the organism. A situation of a "silent" tumor is known, for example, when there is a clinical picture of an oncologic disease but no primary locus can be found (in other words, there exist generalized manifestations but local ones are absent). The loss of weight by oncologic patients long before they are diagnosed (Grosvenor et al. 1989; Kritchevsky et al. 1991) speaks in favor of the same. The tumor growth leads to incompatible with life disturbance in the body homeostasis, which manifests itself in diverse paraneoplastic syndromes affecting almost every organ and tissue of the body (Finora 2003; Kim et al. 2003; Posner 2003; Sato et al. 2003; Tisdale 2002; Yamada et al. 2003).

A solid tumor weighting 1, 10 and 100 g results in initial, expressed and heavy clinical symptoms, respectively. It seems likely that such negligible mass should not have, in itself, any specific consequences, as is the case with benign tumors of even larger mass. Drastic discrepancy between apparent negligibility of the cause and gravity of the effects suggests that the malignant tumor is endowed with a special killer function (Lichtenstein 2005b, 2008). Owing to its universality, this function deserves to be added to the other hallmarks of cancer (Hanahan and Weinberg 2000). The killer function is a resumptive notion, which designates the ability of cancer to kill an organism (no matter how). Its special case is cachexia (the cause of death of ~20% of cancer patients), which is the most demonstrative example of distant and generalized influence of cancer on the body (Tisdale 2002).

The cancer cell is obviously not capable of producing any toxins or doing something that the normal cell is unable to do at some stage of its development (after all, they both have the same genome). Since animal cells produce a host of various biologically active compounds (cytokines, chemokines, ROS, etc.), it is possible to assume the following general mechanism of the killer function: cancer cells, possessing an ordinary set of instruments, use them in unusual combinations and/or concentrations, or at inadequate times and/or place, what brings about incompatible with life disturbances of the homeostasis. For example, the cytokine MIC-1 produced by many tumors is capable of inducing, via central mechanisms, anorexia and loss of weight (Johnen et al. 2007). The cachexia observed in many cancer patients is also due to the factors secreted by the tumor or its environment: (1) increased lipolysis is induced by lipid-mobilizing factor; (2) tissue catabolism is partially mediated by cytokine TNF-α as well as interleukins (IL)-1 and IL-6; (3) proteolysis-inducing factor directly stimulates tissue breakdown (Tisdale 2002). The comprehensive gene expression profiles of each cell type composing normal breast tissue and breast carcinomas demonstrates that extensive gene expression changes occur in all cell types during cancer progression and that a significant fraction of altered genes encode secreted proteins and receptors (Allinen et al. 2004).

The accepted paradigm does not explain the existence of killer function of cancer cell. It is impossible to understand why the appearance of a comparatively small cellular mass, regardless of its type and localization, inevitably kills the entire body (especially in view of the fact that benign tumors may sometimes be as large as several kilograms without deleterious consequences). Death of the body and preceding period of progressive decay are viewed by the accepted paradigm as a self-evident result of tumor growth. But stating a cause–effect relation between two events (tumor growth and body death) is not the same as explaining it. In the accepted paradigm, the indomitable

aggression of cancer cells seems to be both meaningless and counter-productive (for some unknown reason, cancer cells always strive for self-destruction).

The new paradigm offers a simple explanation for this phenomenon: the cancer cells kill the organism because they have been created just for this purpose; the evolution having created these killers has thereby solved some problems of top priority (see above).

Tumorigenesis as differentiation

The notion of a malignant tumor as a specialized organ has received an additional support with the discovery of CSCs (Reya et al. 2001; Shackleton et al. 2009). The concept of CSC traces back, as was repeatedly noted, to the 150-year-old "embryonal rest" theory of cancer by Virchow, Cohnheim and Durante. Based on the similarity between cancer cells and embryonic cells, it is supposed that the tumor arises from stray embryonic cells present in an adult organism. Usually dormant, they are capable of waking up under the influence of various irritants, mainly inflammation, and giving rise to a tumor (Coussens and Werb 2002; Hendrix et al. 2007).

Current molecular and genetic data are also indicative of a similarity between cancer and embryonic cells (Strizzi et al. 2009). Thus, the transcriptional module characteristic of embryonic stem cells is activated in many cancer cells (Wong et al. 2008). Proceeding from Virchow's statement that tumors grow by the same laws that govern embryonic development, the authors of a recent work tried to find out to what degree tumor transcriptome corresponds to that of developing tissues. Indeed, they have observed common global trends of gene expression (Naxerova et al. 2008). A common property of many tumors is the expression of C/T (cancer/testis) antigens (Simpson et al. 2005), which suggests an awakening in cancer cells of the transcription program inherent in germ cells and forming a standard malignant phenotype (immortality, immune evasion, ability to invade and metastasize, genome hypomethylation) (Old 2001).

Based on these data, a theory was advanced according to which a tumor has stem cells which are in many respects similar to normal ones: both possess the ability of self-renewal and differentiation (achieved by asymmetric division), plasticity, unlimited proliferation, ability to recreate the tissue de novo, activity of main regulators of embryonic development (Clarke and Fuller 2006; Hendrix et al. 2007; Postovit et al. 2007; Sparmann and van Lohuizen 2006). Normal mammary epithelial stem cells and breast cancer stem cells display a decreased expression of some specific microRNAs, restraining their clonogenic and tumor-initiating activities, respectively. Apparently, stem cell regulatory pathways are the same as cancer stem cell regulatory pathways (Dirks 2009; Shimono et al. 2009).

The CSC concept assumes the hierarchical structure of a tumor. The most important are CSCs, which make probably a very small fraction of the whole cell population (sometimes, their quantity is substantially higher; Adams et al. 2007; Kelly et al. 2007) and may vary in tumors of different origin (Kennedy et al. 2007). This small subpopulation governs growth of the tumor and its dissemination (Hermann et al. 2007, 2008), its selective eradication is capable, as was shown in a model system, of suppressing the growth of the tumor and its metastases (Gupta et al. 2009a). The bulk of the tumor consists of differentiated non-CSCs destined for final death and unable to recreate a tumor de novo. However, constituting the majority, non-CSCs determine the clinical picture of the disease.

The CSC concept is important in many respects. It allows us to explain difficulties of the present-day chemotherapy (stem cells possess special properties, being, in particular, more resistant to chemotherapeutic drugs) (Gupta et al. 2009a) and aim at new targets. On the other hand, this concept prompts one to reconsider the existing notion about tumorigenesis: to depart from the idea of the chaotic process of trial-and-error type and to consider it as a well familiar cell differentiation—a set of irreversible, deterministic transitions from one stable state to another (namely, the path from pluripotent CSC to differentiated non-CSCs) (Gupta et al. 2009b). As the very notion of "stemness" assumes the ability of the cell to differentiate, identification of the cancer analogue of normal stem cell suggests a corresponding interpretation: just as normal stem cells differentiate into phenotypically diverse progeny with limited proliferative potential, cancer stem cells also undergo epigenetic changes analogous to the differentiation of normal cells, forming phenotypically diverse nontumorigenic cancer cells that compose the bulk of cells in a tumor (Shackleton et al. 2009). In other words, carcinogenesis is probably not "an illness of differentiation" as usually thought, but a special differentiation, during which the cancer cell co-opts standard, ready-to-use modules: the epithelial–mesenchymal transition (Polyak and Weinberg 2009), aerobic glycolysis (Christofk et al. 2008), angiogenic switch (Bergers and Benjamin 2003), immune evasion and immunity suppression (Kim et al. 2006).

The view of tumorigenesis as a differentiation means to some extent the return to the early Virchow's notion. Probably, cancer progenitor cells (Feinberg et al. 2006) are initially present in the body. Staying in a "waiting mode", they are steadily exposed to internal and external mutagens what results in a gradual erosion of the system of checks-and-balances (protooncogenes and suppressor genes) and, eventually, activation of the cryptic "cancer differentiation". The fact that precancerous stem cells are capable of

developing, depending on conditions, in both the malignant and benign directions (Chen et al. 2007) indicates that there are bifurcations in development pathways that route cells along pre-determined trajectories to the alternative states, the normal and cancer phenotypes (Fig. 1). The balance between these alternative pathways apparently depends on association of the stem cell with the niche (Clarke and Fuller 2006; Li and Neaves 2006; Walkley et al. 2007). Mutations, inflammation and other factors can damage this association and favor the choice of the cancer pathway. Indeed, imaging of hematopoietic precursor division in real time has shown that the balance between symmetric and asymmetric divisions is responsive to extrinsic and intrinsic cues and, in particular, to oncoproteins (Wu et al. 2007).

One of the most discussed subjects is the origin of CSC—whether they arise directly from normal stem cells or from cells initially more differentiated but having acquired stem cell properties for the second time (Bjerkvig et al. 2005; Clarke and Fuller 2006; Kennedy et al. 2007; Perez-Losada and Balmain 2003). Apparently, both scenarios can take place. In some cases (bronchioalveolar stem cells, prostate luminal epithelial stem cells, intestinal crypt stem cells), by means of genetic lineage-marking it has been shown that normal stem cells are the cells of origin for corresponding types of cancer (Barker et al. 2009; Kim et al. 2005; Wang et al. 2009; Zhu et al. 2009). On the other hand, recent data obtained on the model of immortalized human mammary epithelial cells (both normal and transformed), have demonstrated an unexpected linkage between the epithelial–mesenchymal transition, a key developmental program, and acquisition of stem cell properties (Mani et al. 2008). Such a backward movement (from more to less differentiated state) suggests a principal possibility of CSC development from non-CSC (Gupta et al. 2009b). This suggestion is in agreement with a hypothesis that stemness is a property of systems, rather than cells. A system with stemness can achieve a controlled size, maintain itself homeostatically, regenerate when necessary and do so by using feedback control. It suggests that under different conditions different cell types can assume the role of "cancer stem cells" (Lander 2009).

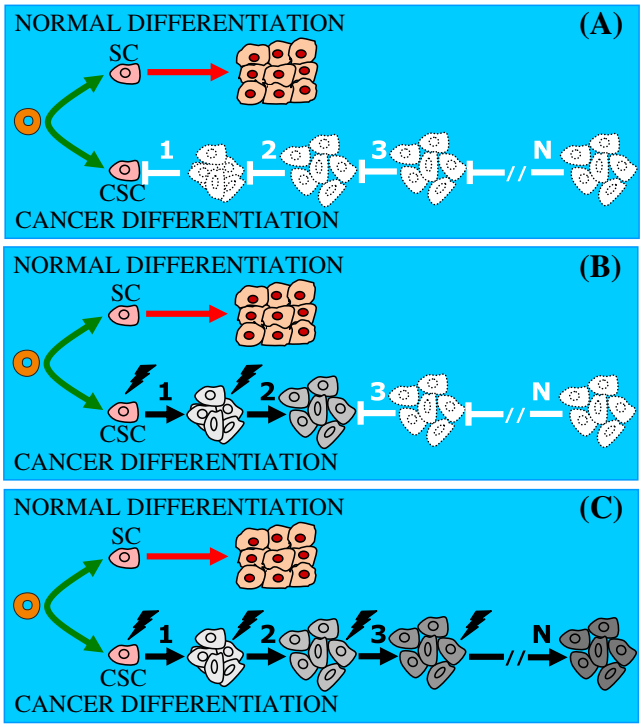

Fig. 1 Normal vs. cancer bifurcations in differentiation pathways. Pluripotent stem cells differentiate to form either normal or cancer stem cells (SC or CSC, respectively). By default, normal stem cell differentiation is active, while cancer differentiation is blocked by several tissue-specific barriers. As the barriers are removed by mutations or some environmental cues, cancer stem cells become gradually activated and the cryptic program is brought into action in a step-by-step manner. As a result, "differentiated" tumor cells are formed. **a** Carcinogenic program is blocked. **b**, **c** Gene mutations sequentially remove the barriers and make "differentiation leaps" possible. The *numbers* denote rate-limiting events (mutations) that are required to pass the differentiation stages. The gradual darkening of cells represents their acquisition of a cancer phenotype. *Lightning arrows*, mutations; *arrows*, passage possible; ⊥, block of cancer differentiation. (Reprinted from A.V. Lichtenstein (2008) Cancer: shift of the paradigm, Med Hypotheses, 71(6):839–850, with permission from Elsevier)

The genetics and epigenetics of carcinogenesis

Two types of changes contribute to carcinogenesis: genetic (damages of the primary structure of DNA—point mutations, deletions, insertions, chromosome instability) (Tomlinson et al. 1996; Wood et al. 2007) and epigenetic (changes in the numerous gene regulatory mechanisms—DNA methylation, chromatin modification, microRNA regulation, mRNA processing) (Baylin and Ohm 2006; Esquela-Kerscher and Slack 2006; Feinberg 2007; Lotem and Sachs 2002; Mayr and Bartel 2009).

Thus, within one process (carcinogenesis), two opposite principles coexist: the chaotic (mutagenesis) and highly regulated (epigenesis). Mutations are local, rare, random, and arise in individual cells (i.e. monoclonal). Epigenetic events, on the contrary, are large-scale, cover the entire genome, coordinate many subsystems, are initiated at embryogenesis and unfold throughout the life (Bernstein et al. 2006; He et al. 2007; Jones and Baylin 2007; Klose and Bird 2006). Epigenetic imprints that lead to cancer are inheritable (Bird 2007), arise early (Fraga et al. 2005), often at the stage of preclinical changes (Jacinto et al. 2007; Sempere et al. 2007), affect simultaneously many cells (are polyclonal) (Feinberg et al. 2006) and have the deterministic nature (Gazin et al. 2007; Keshet et al. 2006; Mayr and Bartel 2009; Widschwendter et al. 2007). The methylation of

promoters of some suppressor genes is not accidental and most likely imprinted in the cell genome (Schlesinger et al. 2007). The complex coordination of epigenetic events suggests their programmed character (Baylin and Ohm 2006; Jones and Baylin 2007). Indeed, recent data suggest that malignant transformation is a highly cooperative process, involving synergy at multiple levels of regulation (McMurray et al. 2008).

As both components are undoubtedly important for carcinogenesis, a question arises of how they relate to each other and what is the role of each of them. Answering this question will allow to find out whether cancer is casual or regular. The prevailing notion that it is mutagenesis that leads to cancer assumes the casual character of the latter. However, in connection with the ongoing reappraisal ("epigenetics wins over genetics"; Lotem and Sachs 2002), there is a feeling that this phenomenon is predetermined. The theory of multistage carcinogenesis and the ensuing notion of transformation resistance of cells as the countdown trigger of carcinogenesis (see above) lay the basis for some assumptions. If one describes cancer differentiation as a pathway with several roadblocks, then mutagenesis would serve as a mechanism of taking those barriers away and step-by-step bringing this cryptic program into action: it manifests itself in outward appearance as successive precancerous tissue lesions (Fig. 1).

The transformation resistance of normal individuals is evolutionary adjusted in such a way as to postpone tumor appearance to post-reproductive period. Since the resistance of individuals with germ-line mutations is decreased resulting in a shortened period of carcinogenesis, they suffer from cancer in the reproductive period of life. This interpretation can, by the way, explain the differences in the spectra of hereditary and sporadic tumors with the same genetic defects (Fig. 2).

Thus, the allocation of functions between mutagenesis and epigenetics during carcinogenesis looks as follows: the former is the driving force of "cancer differentiation", whereas the latter is the mechanism of its implementation (Lichtenstein 2008). The acquisition by the cancer cell of the necessary functional modules is achieved by means of genome reprogramming with the use of epigenetic machinery. The epigenetic memory provides the inheritance of the acquired properties by generations of transformed cells (Ting et al. 2006).

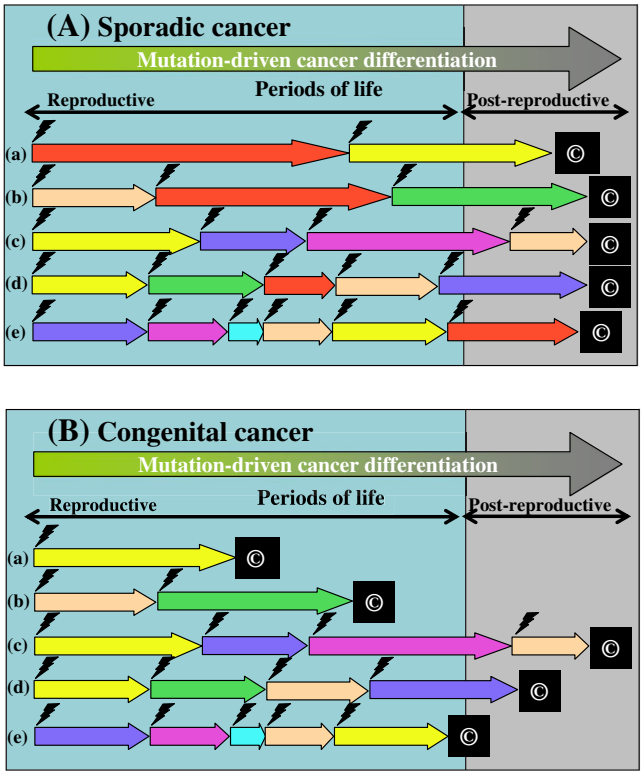

Fig. 2 Mutation-driven cancer differentiation. Tissue-specific transformation resistance of cell (TRC) values are determined, in part, by different numbers of rate-limiting events (mutations, indicated by lightning arrows) required for cancer appearance (denoted with © symbol). Tissues characterized by a low risk of cancer (low mass, low proliferation) are characterized by a low number of rate-limiting events and, consequently, by relatively big "differentiation leaps" shown as differently colored arrows (tissues (a) and (b)). On the contrary, the high risk of tissues with a large mass and active proliferation is offset by a greater number of mutations and a relatively smaller impact of each of them to differentiation (tissues (d) and (e)). **A** The TRC values of different tissues have been adjusted during evolution so that sporadic cancers arise mainly in the post-reproductive period of life (or do not arise at all). **B** A germ-line mutation in specific gene (*red color*) reduces the TRC value of all cells in the body, but to a different degree: to a greater degree in tissues (a) and (b), and to a lesser degree in tissues (d) and (e). In tissue (c), the "red" gene is not involved in carcinogenesis and its mutation is not manifested. As a result, there is a high probability of multiple tumors in individuals of reproductive age, and the spectra of these tumors may differ from those observed for sporadic tumors with the same genetic defect. Germ line mutations of cancer genes are thus subject to the purifying selection. (Reprinted from AV Lichtenstein (2008) Cancer: shift of the paradigm, Med Hypotheses 71(6):839–850, with permission from Elsevier)

DNA methylation

Recent evidence indicates that epigenetic changes might "addict" cancer cells to altered signal-transduction pathways during the early stages of tumor development (Baylin and Ohm 2006). DNA methylation pattern undergoes the most significant changes (Baylin et al. 1998; Esteller et al. 2001; Jones and Baylin 2002; Toyota and Issa 1999). In normal cells, the CpG islands (clusters located in the regulatory sites of genes) are usually not modified, whereas single CpG dinucleotides spread over the genome (80% of the total) are, as a rule, methylated. Tumor cells undergo a reciprocal change: local hypermethylation of some of the CpG islands is combined with global genome demethylation (Bernstein et al. 2007). The CpG islands methylation,

possibly caused by an abnormally high concentration of DNA methyltransferases in cancer cells (De Marzo et al. 1999), leads to gene silencing (a stable inactivation of the adjacent gene) owing to steric hindrances in the binding of transcription factors to the promoter as well as histone hypoacetylation (Stimson and Vertino 2002).

Current data provide evidence for the early occurrence, universality and nonrandom character of local hypermethylation (Costello et al. 2000; Luo et al. 2005; Martin-Subero et al. 2009). This modification is likely to affect genes, which in embryonic stem cells are associated with Polycomb group proteins (these proteins repress cell differentiation genes) (Ohm et al. 2007; Ohm and Baylin 2007; Schlesinger et al. 2007; Widschwendter et al. 2007). This fact supports the view that cancer stem cells originate from normal stem cells, in which the transient repression of differentiation genes by Polycomb group proteins is replaced with permanent gene silencing. As a result, these cells are blocked at a perpetual state of self-renewal (Widschwendter et al. 2007).

Methylation analysis on a genome-wide scale by means of a combination of immunoprecipitation of methylated DNA, microarray technology and bioinformatics has shown that cancer-related de novo DNA methylation comes about through an instructive targeting mechanism. De novo methylated genes possess a number of distinctive properties (they belong to certain functional classes, have common sequence motifs in their promoters and cluster location). Some of them are initially (i.e. still in the normal cells) inactive, what excludes the possibility that these events may be subject to growth selection. This instructive mechanism of de novo methylation in cancer cells is thought to be similar to that occurring during normal development (Cedar and Bergman 2009).

In other cases, promoter methylation and gene silencing confer obvious selective advantages to the cell: for example, silencing of suppressor genes (in particular, *RB1, VHL, CDKN2A, CDKN2B, CDH1, PTEN*) and DNA repair genes (e.g. *MLH1*, *BRCA1*) (Esteller et al. 2001; Jones and Baylin 2002; Karpf and Matsui 2005; Khan et al. 2004) results in uncontrolled proliferation and high variability due to genome instability.

Another prominent feature of cancer cells seen already at early stages of transformation is the global demethylation of the genome (Chen et al. 1998; Rainier and Feinberg 1988). The important role of this epigenetic alteration follows from data that the demethylating agent 5-aza-2′-deoxycytidine can transform cell cultures (Rainier and Feinberg 1988), while chronic dietary methyl deficiency induces hepatocarcinogenesis in rodents (Pogribny et al. 1997). In normal cells, the sequences of compact chromatin are strongly methylated and functionally repressed. Genome demethylation strongly influences chromatin condensation, expression, replication schedule, chromosome stability (Karpf and Matsui 2005; Rodriguez et al. 2006), mutation rate (Chen et al. 1998).

Recent data have complicated the simple scheme of "local DNA hypermethylation—global demethylation" in cancer cells. First, de novo DNA methylation is a non-autonomous process but is tightly intertwined with other epigenetic events: modifications of chromatin (in particular, methylation–demethylation of histones) (Bird 2001; Cedar and Bergman 2009; Ciccone et al. 2009; Esteller 2007; Vire et al. 2006), and with small RNA regulation (Lujambio et al. 2007; Lujambio and Esteller 2007; Moazed 2009). Second, not only *hyper*methylation but also *de*methylation of gene promoters can be aberrant in cancer cells (Smith et al. 2009). These modifications affect functionally different groups of genes, suppressor genes and candidate protooncogenes (*TKTL1*, *H19*, *MAGEA2*, *MAGEA3/6*, *MAGEA4*, *MAGEA11*, *GPR17*, *GRIN1*, *C19ORF28*), respectively. As a result, the former are silenced, while the latter activated. Activation of these physiologically repressed genes takes place in many solid human tumors and is carried out with the participation of transcription factor BORIS (Smith et al. 2009).

Finally, a new approach (comprehensive high-throughput array-based relative methylation) has allowed the discovery of the so-called CpG island shores. They represent tissue differential methylation regions and cancer differential methylation regions (T-DMRs and C-DMRs, respectively), located at some distance (1–2 kb) from the CpG islands (Irizarry et al. 2009). The methylation status of these evolutionary conserved regions (quite similar in man and mouse) controls the expression of the adjacent genes. In colorectal cancer, C-DMRs undergo much more significant changes than the corresponding CpG islands. The fact that methylation changes in cancer are at sites that vary normally in tissue differentiation, is consistent with the epigenetic progenitor model of cancer. The latter proposes that epigenetic alterations affecting tissue-specific differentiation are the predominant mechanism, by which epigenetic changes cause cancer (Feinberg et al. 2006; Feinberg 2007).

MicroRNA regulation

The most significant discovery of last time is the identification of microRNAs, which are involved in negative regulation of gene expression (Carthew and Sontheimer 2009). These short molecules (20–25 nucleotides long) are processed from large precursors (pri-miRNAs and pre-miRNAs) and transported to the cytoplasm. As a part of RNA-induced silencing complex (RISC), they interact with partially complementary mRNA sequences at their 3′-untranslated regions (3′-UTR). The result is translation inhibition or mRNA degradation. A specific feature of this

kind of regulation is its promiscuity (the ability of one microRNA to interact with numerous targets) and, as a consequence, a multitude of effects (Carthew and Sontheimer 2009; Esquela-Kerscher and Slack 2006; Ma and Weinberg 2008). Approx. one thousand microRNAs known today are supposed to regulate translation of about 30% mRNA, thereby being involved in all fundamental processes: cell division, differentiation, apoptosis, etc. For example, microRNA-200c (miR-200c) inhibits the expression of the Polycomb gene *Bmi-1* (this self-renewal factor sustains stem cells in postnatal life) and thus prevents both normal and cancer cells from acquiring stem cell properties (Shimono et al. 2009).

MicroRNAs play a dual role in carcinogenesis: they may be either suppressors or oncogenes. Even the same microRNA may play such a role: for instance, miR-221 and miR-222 target an oncogene, *KIT*, and therefore function as tumor suppressors in erythroblastic cells, but they target a number of tumor suppressors and function as oncogenes in various human solid tumors (Croce 2009). A decrease in certain microRNAs as a result of mutations, deletions or epigenetic silencing may inhibit the expression of suppressor genes indirectly, for example, via ensuing activation of DNA methyltransferase genes. Being important epigenetic mediators, microRNAs are, in their turn, under epigenetic control: promoters of their genes in cancer cells are often methylated (Croce 2009).

A number of observations indicate an instructive character of microRNA regulation in carcinogenesis. For instance, using a mouse model of multistage tumorigenesis, involving the stepwise transformation of pancreatic islet β cells into pancreatic neuroendocrine carcinomas, and high-throughput microRNA profiling, it was found that microRNA dynamics in the stages of tumorigenesis correlate with hallmark capabilities of cancer (i.e. a distinct microRNA expression signature corresponds to each of the investigated stages). An important example is the coupling of metastases with down-regulation of the miR-200 family, which interferes, as was mentioned above, with acquisition by cells of stem cell properties (Olson et al. 2009). Some microRNAs (in particular, miR-10b, miR-31, miR-182) are able to control different stages of formation of metastases (Hurst et al. 2009).

Another striking phenomenon is the widespread shortening in cancer cells of 3′-UTRs of mRNAs by alternative cleavage and polyadenylation (APA) (Mayr and Bartel 2009). This is probably a mechanism used by tumor cells, by which "cancer-associated" genes escape microRNA-mediated silencing (de Lopez et al. 2007). Since shorter mRNA isoforms usually exhibit increased stability and produce tenfold more protein, the high incidence of APA in cancer cells suggests its important role in oncogene activation without genetic alteration.

Perspective

The new vision of cancer as natural phenomenon (programmed death of the organism) has a direct relation to current research, particularly to choosing directions of future investigations and treatment strategies. It explains, on one hand, great difficulties in treating cancer and, on the other hand, brings to the forefront apparently the most important property of the cancer cell, namely its killer function, the mechanism of which remains largely unknown. The situation in oncology is similar today to that in gerontology, where as yet "no hypothesis has emerged that yields a useful definition of dying of old age in terms of cell and tissue biology" and "the accumulated data fail to provide any clue as to the mechanism" (Rando 2006).

Meanwhile, the now existing high-throughput approaches to serial measurement of global gene expression could have offered a general picture of the events that unfold in the tumor-bearing organism. Such research could show what cell systems and metabolic pathways and in what tissues undergo most significant changes under the influence of a growing tumor, and to what extent these changes are universal for various types of cancer. Thereby, it would be possible to get an insight into the biochemistry of the cancer-induced programmed death of organism.

Targeted therapy is now considered to be the most promising approach of combating cancer. It is supposed that "the promise of targeted therapies will be realized as our understanding of cancer biology continues to improve" (Hait and Hambley 2009). In reality, however, the known achievements along this line seem rather an exception than the rule and, moreover, there are serious doubts about the possibility to use targeted therapy as the main and sole means for elimination of cancer (Hambley and Hait 2009). Indeed, the differences between the normal and cancer cells are so small, numerous and evasive that the invention of "a magic bullet" that would hit all cancer cells and nothing except them does not seem feasible. For these reasons, modern chemotherapy is nothing but a medical version of "friendly fire". It seems likely that this situation will remain so until the search of targets will be limited exclusively to the cancer cell itself.

A deeper understanding of the killer function mechanism could help changing the situation. It is most likely that this function is accomplished by means of some peculiarities of the cancer cell secretome (see above). In such situation, an "intercepting therapy" aimed at neutralization of cancer cell rather than its destruction, might be effective. In model systems, this approach has proved to be promising: antibodies to VEGFR1 and VEGFR2 inhibit the formation of metastases in mice, in which VEGFR1- and VEGFR2-positive bone marrow cells participate in formation of premetastatic niche (Kaplan et al. 2005); antibodies to MIC-1 prevent cachexia in mice with xenografted prostate

tumors (Johnen et al. 2007); antibodies to interleukin-23 enhance the immune response and protect the animals from chemical carcinogenesis (Langowski et al. 2006). As the killer function is only inherent, apparently, in cancer cells, its elimination will hardly invoke any strong side effects, this "Achilles' heel" of the present-day chemotherapy.

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Conflict of interest The author declares that he has no conflict of interest.

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