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REVIEW

G. I. Abelev

T. L. Eraiser

On the Path to Understanding the Nature of Cancer

G. I. Abelev* and T. L. Eraiser

Blokhin Cancer State Research Center, Russian Academy of Medical Sciences, Kashirskoe Shosse 24, 115478 Moscow, Russia; fax: (495) 324-1205; E-mail: abelev@crc.umos.ru; eraisert@mail.ru

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Abstract—In this essay crucial problems of the origin of cancer and the development of malignancy are discussed. The problem of precancer and three ways leading to malignancy are considered: induction of tumor precursors, accumulation of genetic traits common for tumor growth, and the role of inflammation in tumor induction. The nature of viral oncogenes and modes of their action are described in the context of their origin as a component of the viral genome. Oncogenes of RNA-containing viruses and DNA-containing tumorigenic viruses are described together with cellular protooncogenes, which are progenitors of RNA-containing viral oncogenes. Hematological malignancies are described as an intermediate form between simple tumors induced by a single oncogene and more complicated epithelial tumors. The roles of tumor suppressor genes and the interaction of several oncogenes in the formation of carcinomas and also the role of progression in tumor evolution are discussed.

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A malignant tumor is an autonomously proliferating immortal cell clone continuously evolving to independence outside the body's control against invasion and metastasis.

The nature of malignant tumors has been most comprehensively studied during the last 50 years. Although it is still far from being clear, it became possible to determine the fundamental mechanisms involved in malignant growth. In 2006, a textbook *The Biology of Cancer* [1] on fundamental oncology was published by Robert Weinberg. This monograph is an attempt to formulate general principles of malignant growth. This attempt has created a basis for understanding and estimation of the trends in studies of the nature of cancer and for determination of the place of a researcher's own work in the general picture of advances to understanding the nature of malignant growth. The present collection of analytical reviews is designed to elucidate separate parts of this picture without erasing its general outlines.

This article is in no way a summary or review of Weinberg's book; it only presents some considerations that appear in the course of reading the book.

ORIGIN OF TUMORS AND THE PROBLEM OF PRECANCER

Monoclonality of tumors clearly indicates that malignant growth is based on single genetic events, mutations (or a mutation) leading to a steady deviation of a cell from the normal program of its development and existence. Mutations are rare and accidental. They have a *frequency of appearance* but have no regular pathway to determine their nature and physiological essence. Just this resulted in the idea that the nature of tumors is unpredictable and their appearance is irregular, i.e. that

Abbreviations: AFP) alpha-fetoprotein; CLL) chronic lymphoblast leukemia; CML) chronic myeloid leukemia; ECM) extracellular matrix; EMT) epithelial–mesenchymal transition; FISH) fluorescent *in situ* hybridization; GSA) group-specific antigen; LOH) loss of heterozygosity; MGCV) mammary gland cancer virus of low oncogenicity; MMP) membrane metalloproteinases; PCR) polymerase chain reaction; RSV) Rous sarcoma virus; VEGF) vascular endothelium growth factor; VIGF) VEGF inhibitor.

* To whom correspondence should be addressed.

there is no regular *precancer*. But pathomorphology of tumors clearly suggests that a morphologically pronounced precancer should exist. According to L. M. Shabad, "Every cancer has its precancer" [2]. How should we combine this idea with monoclonality of tumors? How can we explain the resemblance of precancer with malignant clones arising later, as is often observed?

We can now describe three paths of the appearance of precancer. The first path is an induction and predominant proliferation of precursor cells of certain tumors; the second path is the appearance of genetic changes sharply enhancing the probability of generation of a tumor clone; and the third path is formation of a nontumor tissue, or *stroma*, capable of producing extracellular matrix, growth factors, and factors promoting vascularization of tumors. These paths are considered by Weinberg in detail [1].

Activation of precursor cells. Chronic inflammation is a reliable precancer, as has been convincingly shown for hepatocellular cancer when infection with hepatitis B or C viruses leads to a high probability of development of liver cancer in humans [3]; similarly, infection with *Helicobacter pylori* increases the probability of development of human stomach cancer [4]. In this case activation of tumor precursor cells, stem and committed cells of normal tissue, is the most likely mechanism.

Stem, committed, and terminally differentiated cells. The initial structure for the majority of organs is a stem cell, which is characterized by two traits: the unlimited self-reproduction and capability of several discrete differentiations [5-7]. Stem cells are never exhausted; they are very few in number and usually located in *niches* well protected against external influences [8]. The next step in stem cell differentiation is represented by a *committed* precursor, or an *amplifier* cell, which forms the proliferative compartment of a tissue. The cells of this compartment possess a partial self-reproducibility, are continuously proliferating, and capable of limited differentiating. These cells are sensitive to regulatory factors, such as hormones or growth factors, which regulate their proliferation. The bulk of tumor cells belong to just this stage.

It is not known whether the majority of tumors can arise from a proper stem cell or from a committed precursor which, as a result of mutations, is distinguishable from the stem cell and, gaining the ability for unlimited self-reproduction, becomes a stem cell of a tumor, which fully or partially loses ability for terminal differentiation. This question is crucial not only for understanding pathogenesis of tumors, but also for their treatment [9]. The matter is that most antitumor drugs pointedly suppress enzymatic systems of DNA self-reproduction, i.e. proliferating cells. Because proliferating cells are committed precursors, just they are destroyed first and determine the sensitivity of tumor to radio- and chemotherapy. The same refers to the suppression by hormones (e.g. androgens in mammary gland cancer). But stem cells of normal tissue are beyond the cycle and located in physiologically and anatomically protected niches and, therefore, are less sensitive to radio- and chemotherapy. This creates a gap, which allows antiproliferative exposures to inhibit the bulk of tumor cells without a complete eradication of stem cells of the tumor [10] and of normal tissue. Increases in the concentration of chemotherapeutic drugs or in radiation dose are limited, first of all, by the sensitivity of intestinal and bone marrow stem cells, which are in properties most similar to the proliferative compartment[1].

The increased sensitivity of tumors to chemotherapy, radiation, and growth factor inhibitors is consistent with this viewpoint, as well as limited differentiation of most tumors [9, 10]. Pluripotentiality of the stem cell and the monopotential differentiation of most tumors suggest that tumors originate from committed precursors, which are, as a rule, monopotent[2]. In any case, mono- or pluripotentiality of the majority of tumors is convincing evidence of their origin from different stages of precursor cells. Thus, in CML (chronic myeloid leukemia) genetically determined by the translocation t(9; 22) leading to generation of a new gene *BCR-ABL* (inside the so-called Ph-chromosome), this gene is present in the cells of erythroid, lymphoid, and myeloid differentiation lineages, but leukemia develops only within the myeloid lineage, up to blast crisis which usually covers early stages of both myeloid and erythroid differentiations [11, 12]. Thus, in CML the *BCR-ABL* gene can be determined in the stem cell, but it is realized only in the myeloid differentiation lineage that underlies CML monopotentiality.

But in any case a question arises: How does the tumor precursor acquire immortality? The lifetime of any cell is limited by the number of divisions that it can pass (the Hayflick limit). This is determined by an incomplete replication of DNA during the normal cycle of cell division, which results in shortening of chromosome ends, or *telomeres*. (The bold hypothesis by A. M. Olovnikov [13] already in the early 1970s preceded the appearance of this problem and its study.) Chromosome ends form telomeres that protect them against inevitable sticking together; the telomeres do not carry genetic information but prevent heteroduplexing of DNA, i.e. formation of DNA complexes of different chromosomes. Destruction of telomeres results in sticking together of chromosome ends and death of the cell. Recovery of telomeres and their synthesis catalyzed by the enzyme *telomerase* leads to acquisition by the cell of an unlimited division potential, i.e. immortality. In the normal organism, telomerase is produced

[1] Contemporary chemotherapy searches for inhibitors of a *specific* action of oncogenes or integrated protooncogenes for suppression of tumor growth [9].

[2] In some rare cases, *monopotentiality* is replaced by *oligopotentiality*, as exemplified by acute myeloid leukemia presenting a mixed differentiation, both myeloid and lymphoid [12, 14].

only in stem embryonic cells, which are precursors of spermatocytes and oocytes and are capable of an unlimited number of divisions. When somatic cells exhaust their proliferative potential they die, or *crisis* sets in. The crisis can be overcome only by mutants that recover the synthesis of telomerase, or by cells which retain viability and possess hybrid chromosomes deprived of telomere regions. Thus, the crisis can be survived by very few (unique?) cells, which either gain independence of the telomere destruction, or synthesize telomerase, or possess chromosomal ends independent of telomeres [15].

Overcoming the crisis leads to aneuploidy, which is the main cause of genetic instability of a pretumor and cancer cell and is many times higher than mutation frequency. Genetic instability supplies rich material for progression, which begins even during the pretumor period [15, 16].

The outcome from the crisis associated with changes in the set and structure of chromosomes has no need to be associated with tissue malignization. There are immortal lines of normal cells, for instance, 3T3, which do not produce tumors on *in vivo* injection, but can be easily and routinely transformed by *oncogenes* [15].

Thus, immortality is a necessary but not *exclusive* feature of a tumor cell, or more correctly, of a stem self-reproducing line of tumor cells.

Monopotentiality and immortality present a combination of traits, which are typical but insufficient for a tumor cell. Appearance of these traits is a necessary stage on the path of tissue evolution to malignant growth.

Thus, both a normal stem cell and a committed precursor in their biological features are closest to a tumor stem cell. Induction of just these cells in pathologies must be considered as precancer. This situation is very clearly exemplified by liver disease leading to disorders in its regulation at the cost of replication of mature hepatocytes. Liver poisoning with the alkaloid retrorcine [17] or some chemical carcinogens inhibits division of hepatocytes or their sensitivity to proliferation stimuli. A partial hepatectomy under conditions of suppressed proliferation leads to an outburst of a new formation of hepatocytes from precursors, so-called oval cells, which give rise to young hepatocytes and bile duct cells [5]. Damage to hepatocytes gives a wave of production of the serum tumor fetal antigen *AFP* (alpha-fetoprotein). This wave becomes a subthreshold value as the liver regenerates, but it correlates with a subsequent formation of hepatomas. Moreover, on replacement with a transplanted homologous liver the retrorcine-poisoned liver gives a flash of hepatomas in the transplanted tissue [18]. All these data are consistent with the hypothesis about the origin of liver tumors from the population of oval cells, which are committed precursors of hepatocytes [5, 19]. A similar situation is also observed in lung cancer [20]. It should be noted that single mutations of committed precursors in CML can generate corresponding tumors [21].

Thus, we can consider a tissue damage leading to proliferation of its stem cells and committed precursors as a condition inducing the population of cells (most similar to tumor cells) with a very high risk of producing a corresponding clone.

Genetic predisposition or genetic precancer. It is most likely that a tumor, which is a clone of cells with stable pathological features, is a genetically changed clone, i.e. a result of one or several mutations. Hereditary forms of cancer or of hemoblastoses correspond to this viewpoint. Most demonstratively, hereditary cancer is exemplified by *retinoblastoma*, or the malignant tumor of the ocular retina [22]. This tumor is caused by a recessive mutation inherited from the parents. One functioning copy of *Rb* is sufficient to maintain the normal cellular phenotype, but a sporadic mutation in the ocular retina cells that inactivates the other *Rb* allele leads to appearance of retinoblastoma in early childhood; virtually all cases of hereditary retinoblastoma are characterized by successive damage to both eyes [23]. Consequently, the initial mutation determines the precancer, which is realized in a large population of cells carrying the other mutation.

The nature of the *Rb* gene has been established. A protein product of the *Rb* gene controls the cell cycle passage. The function of the *Rb*-encoded protein (pRb) is regulated through its phosphorylation–dephosphorylation [22]. In the cells beyond the division (G_0 phase) pRb is dephosphorylated. During the G_1 phase, this protein is gradually phosphorylated, and in the hyperphosphorylated state crosses the "restriction point" which separates G_1 from the S-phase, which is the phase of DNA synthesis. Then pRb is dephosphorylated before the beginning of a new mitotic cycle. The phosphorylation activity is determined by cyclin D interacting with mitogenic signals. Mutations in pRb make this protein independent of the mitogenic signals, which determine ceaseless (and therefore unregulated) mitoses of the retina cells that underlie the appearance of retinoblastoma.

Breast cancer is another clear example of the role of genetic changes [24]. Appearance of this cancer is partially controlled by the genes *BRCA 1* and *2*, the mutation frequency of which is in correlation with the frequency of occurrence of this cancer. The nature of the association of *BRCA 1* and *2* with appearance of mammary gland cancer is not established, but genetic factors are very likely to play a role in the appearance of this tumor in some populations.

Another a very demonstrative example of the role of genetic changes during precancer is presented by the behavior of the *APC* gene in the course of development of large intestine cancer: the loss of the *APC* gene function dramatically increases the risk of adenomatous polyposis, which facilitates the development of colorectal cancer clones [25]. However, the *APC* gene mutation alone is insufficient for the appearance of a malignant tumor. Adenomatous polyposis produces a population with a

high risk of development of large intestine *monoclonal* cancer.

The *APC* gene controls a specific adhesion of the intestinal epithelium cells, and disorders in this adhesion are necessary for appearance of polyp but insufficient for appearance of a malignant tumor clone [25][1].

A similar situation seems to occur in CML: the *BCR-ABL* translocation inevitably leads to CML (see above), but not immediately after the introduction of the Ph-chromosome into human cells transplanted into athymic mice, but some time later and only in a few clones of cells [26]. Thus, the *BCR-ABL* translocation sharply increases the risk of appearance of CML but fails to directly result in its appearance. In this case, the appearance of the *BCR-ABL* gene most likely leads to precancer, which determines the further program of development of the tumor.

Summarizing these situations, we can also describe another purely genetic pathway of the appearance of precancer: formation of components necessary for the tumor as *its constituents* but insufficient to induce it. A widely distributed mode of inactivation of tumor suppressor genes also belongs to this pathway. The gene *p53* and related genes control the entry into apoptosis of cells damaged by external agents, mutations, or aging [27]. In addition to serious impact on the regulation of the cell cycle, mutations in this gene induce escape from apoptosis of many cells, genes of which during crisis can acquire immortality and supplement the immortal genotype of a tumor cell.

Early stages in the evolution of tumor precursor cells, when they have no selective advantage but are obviously evolving towards formation of a tumor clone, are still not fully investigated [28].

Thus, various paths of genotype evolution form a typical precancer enhancing the probability of appearance of *definite* tumors.

The role of inflammation in development of precancer. The past decade was a "decade of precancer" in studies of the role of inflammation in tumor growth, although the role of chronic inflammation had been under study much earlier [2]. But here there was a main contradiction: first, inflammation envelops large populations of cells, while tumors are monoclonal and, second, tumorigenesis is underlain by pathology of individual cell mutations which *are not associated* with inflammation.

However, three linking channels have been found between inflammation and malignant growth. These are, first, induction of proliferation of stem cells and mainly committed precursors – amplifiers; then the tumor *stroma* is generated [29], which creates an adequate extracellular matrix for invasion and metastasizing; and, most important, *angiogenesis*, or formation of microcirculation needed for respiration and nutrition of the tumor and elimination of products of its vital activity. Finally, generation of growth factors, cytokines, required for tumor growth [30-34].

Induction of tumor precursor cells. This path is especially expressed in liver. Mature hepatocytes correspond to *committed* precursors. They sense a surgical hepatectomy and accurately recover its lacking part by proliferation. One can repeat hepatectomy many times, and each operation will be accompanied by the regeneration wave from the proliferation of mature hepatocytes. No inflammation features are observed [5]. Consequently, hepatocytes correspond to cell amplifiers: they "feel" the loss of the liver cells, respond by an accurate proliferative reaction, and react to the liver growth factor. Concurrently they function as differentiated cells; they synthesize blood serum proteins and detoxify xenobiotics. The liver regeneration after hepatectomy begins immediately after the operation at the expense of remaining hepatocytes, which are dividing synchronously in accordance with requirements of the recovery of the liver, and this can be repeated several times upon each repeated hepatectomy. However, on chronic liver intoxication with CCl_4 or with resorcine the proliferation of hepatocytes is inhibited and the liver recovers at the expense of proliferation of hepatocyte precursors, which can differentiate into hepatocytes and cholangiocytes. These are the so-called *oval* cells marked with the fetus-specific protein AFP. Consequently, in this case the conditions are favorable for proliferation of newly formed precursors of tumor cells. One way or the other, a situation of precancer arises in the liver even before formation of a tumor clone. Probably such a situation is also produced in a two-stage carcinogenesis, when an *initiator* induces a mutation, and a *promoter* promotes the realization of this mutation and transformation of the mutant cells into a tumor.

There are well-known oncogenic effects of the hepatitis B and C viruses, which are responsible for appearance of groups with high risk of liver cancer, especially if they are combined with the hepatic toxin aflatoxin [3]. These viruses have the same indirect mechanism of the oncogenic effect mediated through chronic inflammation and stimulation of precursor cells. In other words, here we meet a situation of *precancer* associated with inflammation. No doubt, production of specific growth factors, cytokines, contributes to formation of precancer [35] and also of vascular endothelium factors (angiogenesis) [36].

Macrophages possessing multiple functions are generated and accumulated in the inflammation focus. The functions of macrophages include production of colony-stimulating factors, cytokines stimulating fibroblast proliferation, and of metalloproteinases destroying the extracellular matrix collagen, which is a basis of oriented cell growth (basal membrane) [37].

For the growth, a tumor requires provision with oxygen and nutrients, which is achieved by creation of

[1] The *APC* mutation leads to activation of β-catenine, which activates cadherin involved in intercellular contacts.

microcirculation. Hypoxia, which arises on damage of the microcirculation network, always occurs on the damage of normal tissues, and also regularly appears in tumors beginning from their size of 0.2 mm need the development of additional network of blood supply for compensation. Such a network is built due to VEGF (vascular endothelium growth factor) and VIGF (VEGF inhibitor). VEGF is secreted by different tissues, including normal connective tissue and epithelium, in particular, the tumor epithelium [38]. Normal tissues are involved in the tumor *stroma*, which closely envelops the tumor and promotes its growth. Generation of chronic inflammation, production of cytokines, VEGF/VIGF, secretion of MMPs (membrane metalloproteinases), destruction of the extracellular matrix collagen are different manifestations of the stroma activity which ensure conditions favorable for the tumor growth and thus contribute to formation of *precancer*. Thus, formation of the tumor stroma from normal cells creates conditions necessary for formation and growth of tumors, and these conditions may be considered as those of *precancer*.

Summarizing, we conclude that induction and accumulation of tumor precursor cells, accumulation of genetic changes in the tumor cells together with formation of the stroma favorable and necessary for the tumor growth creates a new reliable target for antitumor therapy, which is specific for each tumor and often detectable owing to serological markers or pathophysiological features. Such a target is reasonable and reliable and characterizes just the *origin* of a tumor and not a mature tumor clone.

ONCOGENES AND PROTOONCOGENES

A concept of the *oncogene* appeared in the early 1970s. At first, a temperature-dependent mutation of an oncogenic virus leading to *in vitro* transformation only at the *permissive* temperature and restarting the normal growth at the *non-permissive* temperature clearly indicated the existence of an oncogenic mutation [39]. In fact, the discreteness of the tumorigenic function and its obvious dependence on temperature corresponded to the concept of a temperature-dependent mutation of *a single* gene controlling synthesis of one temperature-dependent protein. Consequently, *a single* gene was responsible for the *in vivo* transformation even of such a highly malignant viral tumor as Rous sarcoma. Intensive studies of tumorigenic retroviruses have revealed a whole family of oncogenes with different activities and action mechanisms, from MGCV (mammary gland cancer virus of low oncogenicity) of mice to RSV (Rous sarcoma virus). Oncogenes demonstrated an elementary carcinogenesis determined by a *single* mutant gene [40]. The oncogenes were peculiar *only* to oncornaviruses, i.e. tumorigenic viruses with the genome represented by RNA. These viruses build a DNA copy of its genome by reverse transcription, and the resulting copy incorporates into the cell genome and becomes a part of a chromosome. Viral RNA is produced on the basis of cellular DNA. If this DNA is replicated from the DNA fragment adjacent to the proliferation-controlling gene, this gene can be captured by RNA polymerase during the synthesis of viral RNA and thus be included in the viral genome. During synthesis of the virus, this gene "returns" into the cell genome but already into another position and goes out of the "normal" gene control. In the early 1970s A. D. Altstein was the first to very clearly formulate a hypothesis about the capture by an oncornavirus [41], and soon this capture was shown experimentally.

Cellular genes capable of incorporating into the genome of oncornavirus and inducing an autonomous (unregulated) proliferation were called *protooncogenes*. Obviously, a viral oncogene arising on the basis of a protooncogene can be formed only within an oncornavirus. Oncornaviruses inducing tumors [39] and carrying oncogenes originating from protooncogenes are most widely distributed in chickens and mice.

The widespread occurrence of pathogenic oncornaviruses in chickens and mice, along with their absence in related species (i.e. in quails and hamsters), is a striking phenomenon. Leukemia oncornaviruses, similarly to bovine leukemia virus BLV, feline leukemia virus FeLV, and human virus HTLV-1 [42], are of another nature and contain no cellular protooncogenes [42].

A widespread, virtually absolute, occurrence of infectious oncornaviruses has been found in chickens and mice [39]. Avian leukemia virus ALV was shown to act as a helper virus for oncornaviruses in chickens [39].

Virus of mammary gland cancer [MGCV] in mice acts as their own gene inheritable by the Mendelian scheme, i.e. as an endogenous virus [43].

We have found that during all stages of ontogenesis, *all* mice are carriers of group-specific antigen (GSA) of leukemia viruses the most closely related to GSA of Gross leukemia virus [39].

Thus, the oncogene of RNA-containing viruses of animals (mice and birds, see below) is a cellular gene (protooncogene) incorporated into the oncornavirus integrated with the cellular genome [40, 44]. Obviously, the oncogene of oncornaviruses is *a single* gene, which occurs *only* in RNA-containing tumorigenic viruses.

Cellular protooncogenes of lowly oncogenic slowly acting oncornaviruses are activated by an insertion oncogenesis mechanism when the genome of oncornavirus incorporates next to the protooncogene and activates it. In this case, the protooncogene acts as a viral antigen from outside of the viral genome. Such a mechanism has been shown for MGCV of mice [40].

A tumorigenic DNA virus cannot capture a protooncogene and is free of classic oncogenes. Genes responsible for tumors induced by DNA viruses have another mechanism of action and another origin (see below). Their tumorigenic activity is not determined by

the cellular protooncogene incorporated into the virus structure.

Oncogenes are structural components of oncogenic oncornaviruses and can be identified and characterized by comparing them with the structure of the initial (non-oncogenic) viruses. In such a manner the main groups of viral oncogenes were established (*SRC*, *RAS*, *ABL*, *MYC*, *SIS*) and shown to occur on different stages of signaling pathways, from growth signal specific receptor, such as PDGF (*SIS* oncogene) to oncogenes acting inside the cell (*RAS*, *SRC*) or within the nucleus (*MYC*) where they activate a specific gene group as transcription factors [44]. The signal specificity is determined by changes in the conformation of all preceding links activated by their ligands, and these changes promote manifestation of the phosphorylating activity succeeded by transmission of the signal (phosphorylation) along the chain until activation of a specific transcription factor interacting with nuclear chromatin. As a result, the viral antigen located in the signal transduction chain and not needing an initial ligand (growth factor or hormone) generates mitogenic signals into the nucleus and thus determines the autonomous proliferation of the cell. Signals from oncogenes are *dominant*. They need no homozygosity because the viral oncogene is beyond regulation as not being a part of the normal genome. Certainly, it should be remembered that the transmission of a mitogenic signal from the oncogene into the nucleus includes many intermediate stages and crosses other signaling pathways. But here we would like to emphasize the role of *a single dominant signal* on the apex of generation of mitogenic impulses [45].

Tumorigenic DNA viruses act by another mechanism. Their genome, or more accurately, individual genes of the genome and products of these genes, such as the large T-antigen (LT antigen) of oncogenic papovavirus, combine with a cellular protein inhibiting the cell proliferation and involved in the regulation of proliferation, inactivate this protein and create an autonomous unregulated proliferation. Target genes, which determine synthesis of corresponding proteins, were called *tumor suppressor genes*. These genes were discovered in the course of studies of oncogenic activity of DNA viruses [46, 47]. Such a mechanism was found for *papovaviruses* (papilloma, polioma, SV40) and *adenoviruses*, and, obviously it is quite different from that of oncornaviruses.

Viral antigen functions as a *dominant* gene and, as a rule, needs no independent activities of other regulatory genes for functioning.

Summarizing, we can see that tumorigenic activity of oncogenic viruses create an *autonomous continuous cell proliferation*, which is a major feature of tumor growth.

The subsequent evolution of a tumor is a result of selection from the genetically heterogeneous cell population leading to invasion and metastasis.

The cells proliferating under the influence of a viral oncogene, similarly to normal cells, exhaust their proliferative potential and come into crisis; upon overcoming the crisis, the cells acquire immortality and, as a rule, enhance their genetic heterogeneity already as fully malignant cells. However, tumors induced by viral oncogenes are the simplest tumors determined by *transfection* of a single dominant oncogene, e.g. *SRC*, *MYC*, or *ABL*.

Introduction of a protooncogene into oncornavirus and insertional mutagenesis are not the only pathways for activation of this gene. Amplification of protooncogenes resulting in their unregulated activation is another pathway. This pathway is rather rare, and is exemplified by pediatric neuroblastoma. Translocation of a protooncogene under an actively expressing gene in this tissue and production of chimeric genes is a much more frequent pathway. This pathway is more similar to the first one, because here the oncogene is activated directly, without involvement of intermediate components. The activated oncogene is always dominant and directly expressed. This is especially pronounced in leukemias, where the cells behave more independently, and the role of cell interactions is lower than in epithelial tumors. Moreover, multicomponent carcinogenesis is rarer and less pronounced in leukemias.

Hemoblastoses. Turning from oncornavirus-induced tumors to spontaneous tumors or those induced by viruses, which do not mediate the transmission of a protooncogene, we shall first consider hemoblastoses, or tumors of the hemopoietic system. From our viewpoint these tumors are the most related to tumors caused by oncornaviruses which act as "carriers of protooncogenes". These tumors are caused by *one* oncogene, a protooncogene (i.e. their own gene), activated by translocation under the promoter of a physiologically active gene, or by mutation of *one* protooncogene. In most cases (if not always), they are dominant, and all known manifestations of such oncogenes are dominant or codominant, e.g. BCR (B-cellular, i.e. immunoglobulin receptor)–ABL or IgG-MYC. In any case, translocations leading to the most widespread leukemias and lymphomas detectable by routine or highly sensitive methods, such as FISH (fluorescent *in situ* hybridization) or PCR (polymerase chain reaction), are located only in *one* chromosome, whereas the morphology of the twin chromosome is normal [48]. The oncogenic effect of an activated protooncogene is obviously dominant. This is responsible for a fundamental difference of *human carcinomas and hemoblastoses*. Human carcinomas are *always* or in the *great majority of cases* induced by *usually recessive* tumor suppressor genes and combined oncogenic actions of several protooncogenes, whereas in the case of hemoblastoses a *single* activated dominant protooncogene is acting, which needs involvement of additional genes only to enhance the effect, as a rule, during tumor progression. Hemoblastoses do not require additional oncogenes for malignization. Leukemia is invasive according to its normal nature and is capable of metastasizing into normal tissue without additional mechanisms

for inducing blood vessels—they are formed during the normal differentiation of hemopoiesis in "explants". The path of hemoblastoses to malignancy is much shorter and easier than the path of carcinomas; therefore, it is not surprising that carcinomas usually require interaction of a *number* of independent oncogenes [49].

Activation of protooncogenes due to translocation frequently occurs in hemoblastoses, and is classically exemplified by translocation of the gene *ABL under the gene BCR promoter* (Ph-chromosome) in chronic myelocytic leukemia. However, in hemoblastoses it is not always clear how, when, and in what cell population the crisis comes. Probably the entry into the blast crisis after a long-term (four to five years) chronic period in CML is just a result of a natural passage into the proliferation crisis of committed myelocytes or proper hemopoietic stem cells. In any case, transfection of cells with CML constructs containing an activated gene of the proper stem cell leads to acute leukemia, which is an analog of blast crisis [14].

Obviously, hemoblastoses are more elementary systems than carcinomas, which, in particular, are determined by direct intercellular interactions (not mediated through cytokines), direct interactions with extracellular matrix, and by acquisition of invasion and metastasis via selection for autonomicity, which is an essence of progression. And another fundamental distinction should also be mentioned: hemoblastoses retain the normal phenotype of the cell precursor as a mechanism involved in protooncogene activation [12, 50].

These features make hemoblastoses extraordinarily similar to the precursor cells; their malignization is especially clear and embossed and can be easily analyzed experimentally.

We have already said that invasion and metastasizing in hemoblastoses are rather retention of normal traits of the hemopoietic tissue than newly acquired features as occurs in the system of carcinomas. In fact, upon maturation hemopoietic cells are not linked to one another by direct contacts, they can penetrate into normal tissues owing to their natural features, as occurs in the case of lymphocytes, neutrophils, macrophages, or NK-cells. Hemoblastoses do not need to form a specific microcirculation network, and production of their clones is not suppressed by the normal microenvironment. Their numbers in the microcirculation is "perceived" by bone marrow precursors, and in the case of hemoblastoses the tumor cells (but not normal ones) have to lose this "perceptibility". Just this seems to cause the inhibition of normal hemopoiesis in leukemia, which is a main feature of progression in hemoblastoses. This underscores once more an "elementariness" of hemoblastoses and their fundamental distinction from carcinomas. The differentiation characteristics of hemoblastoses are very seriously different from those of carcinomas. When carcinomas can more or less lose features of their tissue differentiation, hemoblastoses scrupulously retain their differentiation until the transformation stage. The transformation seems to correspond to "freezing" of the differentiation, and this allows us to finely classify hemoblastoses and determine their origin. This occurs because the overwhelming majority of hemoblastoses are a result of chromosomal translocations, when the role of an "activating" gene is played by the gene actively expressed in the tissue under consideration (e.g. *BCR*), and the differentiation block is determined by an unrelated "activated" gene located on a fragment of the translocated chromosome (e.g. *ABL*). This unrelated gene acts as an "oncogene", which inhibits the differentiation and determines a pathologic autonomous proliferation. It is *single and dominant*, i.e. acts as an oncogene. Additional mutations can enhance or accelerate its action but are not components of its oncogenic effect. Thus, CML can be induced in mice with the gene *BCR-ABL* [25], and transfection of committed hemopoietic precursors with the gene of hemopoietic stem cells leads to induction of acute lymphatic leukemia in mice [14]. This underscores once more a resemblance of hemoblastoses to viral tumors caused by oncogene-carrying oncornaviruses.

The majority of B-cellular hemoblastoses arise on the basis of translocations of different cell genes under promoter of immunoglobulin genes (IgH, Igκ, or Igλ) or the closely related genes of B-cellular receptors (*BCR*) [51]. These translocations "use" genetic recombination mechanisms, which are widely represented at the assemblage of V, D, and J-regions of the H- and L-chains of the Ig molecule and even of hypermutations arising on switching over of Ig classes during production of the memory cells. There is a striking resemblance between mechanisms of normal differentiation and those leading to hemoblastoses in this system.

It should be emphasized that many T-cellular hemoblastoses can also arise as a result of translocations under the T-cell receptor promoter [6, 50].

TUMOR SUPPRESSOR GENES: THEIR ROLE IN THE ORIGIN OF CARCINOMAS

Human retinoblastoma was the first clear example of a gene controlling carcinogenesis (see above). The *Rb* gene is the most distinct and genetically determined suppressor gene. How is its suppressor effect displayed? Studies of the molecular mechanism of its action have shown that the gene itself prevents the entrance of the cell into the G_1/S phase, whereas the mutation of this gene (in the homozygous state) promotes it, i.e. stimulates cell proliferation. Overcoming the G_1/S barrier becomes uncontrolled, does not require a special signal, and the cell becomes autonomous [21]. Moreover, the normal cell "hampers" the passage across the G_1/S barrier and thus acts as a suppressor. The mutation of *Rb* creates an autonomous proliferation of the epithelium, which is a main component of tumor growth. All other specific fea-

tures of tumor underlying the progression can arise (or not arise) as secondary ones, not directly determined by the *Rb* gene. In this respect, functions of *Rb* are limited rather accurately. Its suppression in homozygosity is typical for human tumors.

Gene *p53* is another example of the most universal suppressor gene with parallel functions [26, 52]. The main function of gene *p53* is rejection of cells with a damaged system of DNA replication. Cells with damaged DNA form a complex of the *p53* protein with DNA, and this directs the cells into apoptosis. Another function of *p53* is to slow proliferation on passing the G_0/G_1S block. At this stage, *p53* acts as an anti-oncogene. Inactivation of *p53* leads to survival of tumor and pretumor cells and thus to survival of the tumor clone.

The *p53* system is characterized by a specific sensitivity to stress: different stress exposures induce synthesis of a family of proteins interacting with stress-modified peptides and promote their proteolysis in proteosomes (ubiquitinylation).

The down-regulation and suppression of apoptosis lead to a concentrated entrance of the cell population in the crisis and increase in the number of anomalous mitoses, which dramatically enhances cell heterogeneity with subsequent selection of autonomous variants. Thus, inactivation of the normal function of *p53* results in enhancement of progression and, consequently, stimulates carcinogenesis.

Executing this function, *p53* acts as an antagonist of a nuclear trans-factor, the oncogene *MYC* [26]. The *p53* family also includes proteins with a similar function and genetic control, which regulate the cell entrance into the cycle. Inactivation of this family is a common recessive component of human epithelial tumors, which occurs about five times more frequently than the involvement of protooncogenes.

The usual inactivation of tumor suppressor genes is manifested by the loss of genetic heterozygosity (LOH), i.e. the loss of a chromosome region carrying the corresponding gene, which controls genetic disorders during pathological mitoses [46]. Thus, similarly to the case of *Rb*, the inactivation of this system leads to autonomous proliferation as a main component and to increase in the genetic heterogeneity as a necessary prerequisite of the subsequent progression.

Concluding this section, we would like to emphasize once more specific features of tumor suppressor genes and their role in carcinogenesis:

– first, manifestation of these genes, as differentiated from oncogenes, requires homozygosity for functioning. The loss of the gene in LOH has the same effect as homozygosity;

– second, in some cases suppressor genes *inhibit* the effect of oncogenes and send the oncogene-carrying cell into apoptosis or suppress the oncogene-caused proliferation;

– third, mutant suppressor genes of carcinogenesis are involved in carcinogenesis (epithelial) more frequently than oncogenes;

– fourth, in humans, carcinogenesis usually includes inhibition of suppressor genes;

– fifth, the role of suppressor genes in the origin of hemoblastoses is markedly lower than in origin of carcinomas. It is likely that some hemoblastoses arise *only* upon activation of oncogenes.

TUMOR PROGRESSION

Precancer and transformation lead to the major element of malignant growth—autonomous proliferation and cell immortality. But still it is not a malignant tumor while the tissue does not go beyond limits of its territory or prevents the development of its own genes. Proper malignancy, i.e. invasion and metastasis, as well as dedifferentiation arise during evolution or *progression* of the tumor. The progression of hemoblastoses and carcinomas seems to be different.

Hemoblastoses. In the system of hemoblastoses, progression leads to blast crisis and suppression of normal hemopoiesis, as considered earlier.

Blast crisis is equivalent or nearly equivalent to mutational transition from chronic phase of the disease to phase of *acute leukemia* associated with dedifferentiation, accumulation of immature forms in the bone marrow and liquid blood, and such forms violently proliferate and resemble stem hemopoietic cells carrying the membrane antigen CD34. Transition to blast crisis is especially demonstrative in the evolution of CML and CLL (chronic lymphoblast leukemia).

Carcinomas. Tumor suppressor genes of the *p53* family are the most typical for carcinogenesis of epithelial tumors, and the main function of *p53* is sending into apoptosis of cells expressing mutant genes. Therefore, accumulation of genetic heterogeneity is the most natural specific feature of carcinomas. Genetic heterogeneity is the basis of natural selection for autonomicity and increase in it, which occurs in the population of tumor cells and determines the tumor dynamism. In addition to inactivation of *p53* and related suppressors of apoptosis, the passage of the tumor population through the crisis is a powerful source of cytogenetic heterogeneity presented by disorders in chromosome balance and various chromosome aberrations [15]. These factors are rather distinctly expressed in tumors.

Earlier, we have considered tumors induced by a single oncogene of oncornaviruses or non-viral hemoblastoses also induced by a single oncogene activated or generated as a result of chromosome translocation.

Carcinomas are specified by multicomponent carcinogenesis with involvement of a number of different oncogenes. They seem to be involved during different

periods of the tumor development and determine either different stages of the tumor progression (beginning from precancer) or different levels of malignancy, such as polyps, carcinomas *in situ*, invasive cancer, and metastatic cancer. Multiplicity of oncogenic effects and also the involvement of various oncogenes determine different paths and different results of the tumor progression. Multiple forms of colorectal carcinoma [24] and mammary gland carcinoma [49] are characteristic traits of such a diversity of the progression paths.

The tumor stroma consisting of tumor-associated fibroblasts, vascular endothelium, cellular elements of inflammation, and the basic unstructured substance of connective tissue is very important if not the leading factor of tumor progression. Fibroblasts produce the basic substance, which envelops the tumor—type IV collagen and laminin of the basal membrane, which "supports" the tumor epithelium cells and separates the epithelium from other tissues. The basal membrane is a part of ECM (extracellular matrix) and mainly determines polarization of the epithelium cells, which is the most important feature of their differentiation. The normal epithelium cell "feels" the basal membrane by means of special transmembrane receptors, or integrins. Integrins interact through their extracellular domain with the basal membrane and fibronectin of the ECM and transmit the specific signal into the cell [53]. The tumor cells retain their epithelial behavior and morphology during the period of integrin "work". The loss of integrins in the course of selection for autonomicity and destruction of *cadherin* in early stages of progression, termination of cadherin synthesis as a result of genetic block [54] or of epigenetic block of the corresponding promoter, or cadherin destruction by metalloproteinases associated with the tumor and produced by its stroma lead to dissociation of intercellular contacts. These contacts create the tissue, and their dissociation results in disorganization of the tissue. An organized tissue restrains the autonomous proliferation of tumor, so the selection for autonomicity works against the epithelial organization of the tissue. The epithelial organization of the tissue is maintained by the cell contacts with the matrix, and destruction of this interaction either because of inactivation of integrins or as a result of destruction of the ECM unstructured substance by metalloproteinases leads to depolarization of the tumor cells. And this is associated with inhibition of the master gene *HNF4*, which controls trans-factors of liver differentiation [54-57].

Thus, the events occurring during tumor progression lead to destruction of the epithelial tissue structure and the loss of polar morphology of the epithelial tumor cells [55, 58].

We think that the pivotal event in tumor dedifferentiation is a disturbance of interaction of the epithelial tumor cell with the extracellular matrix—the basal membrane and unstructured intercellular substance, the proper ECM.

Evolution of the tumor stroma is essentially responsible for the above-described events. The production of metalloproteinases by the stroma leads to destruction of the basal membrane and collagen components of ECM. Destruction of the basal membrane with retention of the ECM unstructured substance is a main condition of invasion, when tumor cells retaining the connection with the basic population are spreading beyond limits of the basal membrane and inculcate into territories of other tissues.

Metastasis which, on one hand, spreads the invasion far beyond the initial tissue limits and, on the other hand, rests on the microcirculation system also strongly depends on the stroma, and not only due to destruction of the basal membrane. A tumor cannot grow without a supply of oxygen and nutrients. Hypoxia developing in the region (microregion!) of the tumor development and metastasis disturbs the production of the vascular growth factor (VEGF), which stimulates generation of the microcirculation system in both the tumor tissue and the stroma (!). Induction of proliferation of vascular epithelium cells is necessary for formation of capillaries, and the capillary network is due to activity of the tumor stroma more than to activity of the tumor cells.

Thus, the tumor stroma provides for the existence of the tumor itself and determines the limits of its spreading in the body, as well as development of its distant microfoci. There are data, or hypotheses for the time being, indicating that the long-term retention and renewal of micrometastasis growth depend on behavior of the microcirculation network that supplies these tumor microfoci with oxygen and nutrients. But this does not restrict the role of the stroma in tumor development. Appearance of necrosis and development of a local inflammation promote accumulation of lymphocytes, neutrophils, and macrophages actively synthesizing inflammatory mediators. These mediators include a whole family of substances, which enhance inflammation (the complement system), activate functions of macrophages (tumor necrosis factor), and growth-stimulating factors (cytokines), which stimulate growth of the tumor proper.

Accumulation in the tumor of natural resistance factors (macrophages, normal killers, and T-lymphocytes) responsible for specific control of the tumor growth creates an opposite effect and enhances the natural selection of variants that are insensitive or resistant to immunological control of the tumor growth and, thus, promote further evolution (progression) of the system.

Finally, the carcinoma evolves towards escaping the control of the epithelial structure which, in particular, depends on the presence of the basal membrane in the epithelium. The loss of specific characteristics of the epithelium including the tissue structure, cellular interactions, control by specific growth factors, and acquirement of mobility and morphology of fibroblasts constitutes the so-called *epithelial–mesenchymal transition* (EMT) [59].

The EMT is inherent in the normal epithelium during its development, especially during its early stages, i.e. the gastrulation when the epithelium gains mobility and is actively instilled into the layers located below. The EMT occurs in transient damages of tissue, and epithelial cells concurrently lose polarity, stop synthesis of cadherins, produce vimentin and fibronectin, and acquire mobility. They cease synthesis of cellular nuclear trans-factors and production of antigens specific for epithelial tissues. Epithelial cells become typical fibroblasts. The EMT seems to support invasion and metastasis: epithelial tumor cells become mobile and capable of settling into different territories of the body. It is very important that this transition of cells is *physiological* and not *genetic*, because EMT is *reversible*. Metastases arising on the basis of EMT can acquire morphology of the initial tumor, whereas the epithelium in the wound edges can acquire features of fibroblasts. The EMT is induced on interaction of tumors expressing the *Ras* oncogene and TGFβ. But, in any case, EMT seems to be the terminal stage in progression of an epithelial tumor, when it looses epithelial traits, such as cell polarity, specific cellular contacts, characteristic morphology, and tissue-specific antigenic structure, alongside a simultaneous gaining of traits of fibroblasts, such as expression of vimentin, mobility, and independency of the growth territory.

It is suggested that understanding this process and factors involved in it will provide a basis for a rational therapy of invasion and metastasis, which are major features of malignancy. But what will be in the future is still unclear. The progression has to be unlimited, but EMT seems to be its termination.

Specific features of tumors considered in the present paper allow us to describe general contours of events: through different forms of precancer, generation of oncogene-carrying oncornaviruses, and tumorigenic activity of oncogenes.

Then oncogenes are activated by translocation of protooncogenes under an actively functioning gene, and this is a general mechanism of production of hemoblastoses that combines them with tumors induced by oncornaviruses. Hemoblastoses are a transitional form from tumors of mice and birds to human tumors. Carcinomas arise with a necessary involvement of tumor suppressor genes and usually have a multicomponent carcinogenesis based on *several* activated oncogenes successively contributing to carcinogenesis.

And finally, a novel, broader view on tumor progression may be formulated which includes the precancer stage as its start and the epithelial–mesenchymal transition as a basis of invasion and metastasis at its terminal stage. Such a view suggests a set of new problems to be investigated, in particular, transformation mechanisms of mesenchymal tumors (sarcomas) and their place among human tumors induced by viral oncogenes, hemoblastoses, and carcinomas. What is the role of suppressor genes in these tumors?

Human carcinomas arise with an indispensable involvement of tumor suppressor genes and of genes involved in appearance of precancer. The origin of carcinomas is inseparable from progression, which begins by activation of precancer factors, e.g. proliferation of tumor precursor cells or tumor-specific genetic changes which inevitably include inactivation of suppressor genes, in particular via LOH, and activation of at least two protooncogenes. Inactivation of suppressor genes, first, abolishes the block of proliferation control, and second, suppressing apoptosis promotes accumulation of mutants, i.e. enhances the tumor genetic heterogeneity, which is a material necessary for progression towards malignancy.

Naturally, the fundamental picture of carcinogenesis has large white spots, as follows: mechanism of tumor cell normalization by normal microenvironment [60]; and the *time lapse* between the introduction of the oncogene into the cells and its effect.

These are only some problems for further studies of carcinogenesis.

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