Biochemistry (Moscow), Vol. 65, No. 1, 2000, pp. 107-116. Translated from Biokhimiya, Vol. 65, No. 1, 2000, pp. 127-138.
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REVIEW

Differentiation Mechanisms and Malignancy

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Received September 17, 1999

Abstract—This review considers the relationship between differentiation mechanisms and the genesis and maintenance of tumor phenotype. To a certain extent, carcinomas preserve differentiation markers of normal tissue, and hemoblastoses precisely reflect the direction and differentiation level of their precursor cells. Both tumor types retain the ability to differentiate. Mechanisms of T and B cell differentiation are reviewed considering the activation of protooncogenes by translocation to the region of tissue-specific genes including the immunoglobulin (Ig) and T cell receptor (TCR) genes. Apart from the classical oncogenes (MYC, PRAD, BCL-2), heterologous differentiation of *trans*-factors can be activated in a similar manner. Their activation at inappropriate time and place induces oncogenic transformation in a number of hemoblastoses. Chimeric genes and fused proteins are analyzed, including their genesis by specific translocation resulting in transformation and their role in differentiation and maintenance of the tumor phenotype. Induction of terminal differentiation in leukemia can have significant therapeutic effect. These hemoblastoses include hairy cell leukemia, promyelocytic leukemia, and in part chronic myeloid leukemia. Specific attention is given to the role of intercellular interactions in the control of tumor growth and maintenance of a differentiated state of the cells. It is suggested that alterations in these interactions during tumor progression simultaneously stimulate malignant growth and decrease differentiation level, thus inducing re-expression of embryonic antigens in the tumors.

Key words: differentiation in tumors, translocation in hemoblastoses, tumor progression, extracellular matrix, re-expression of α-fetoprotein in hepatomas

DIFFERENTIATION OF CARCINOMAS

Altered histological type associated with more or less pronounced loss of tissue specificity is characteristic for epithelial tumors (carcinomas). This suggested the hypothesis that tumors are more similar to each other that to a normal precursor tissue [1] and also suggested the concept of antigenic simplification of tumors [2]. However, both theories have a number of exceptions that significantly influence the state of the art. Detailed analysis of the antigenic structure of hepatic tumors (hepatomas) indicates that these tumors never lose tissue-specific antigens completely and always preserve some features of the parental tissue [3]. The tissue-specific antigens can be almost completely preserved in highly differentiated hepatomas, whereas they are only partially preserved in moderately differentiated hepatomas and are completely lost in low differentiated (anaplastic) tumors. In low and moderately differentiated hepatomas, the synthesis of embryonic α-fetoprotein (αFP) begins. αFP is a tissue marker of embryonic liver [4]. αFP was the first immunological marker of carcinomas, and later a whole family of tumor markers was discovered. The appearance of these proteins in the blood is a diagnostic criterion of a certain tumor or a group of similar tumors [5].

Detailed study of the tumor markers has demonstrated that all such markers are differentiation antigens, i.e., tissue-specific proteins or glycoproteins persisted in a certain tumor. These markers are indicators of tumor formation and progression [5]. Preservation of direction and level of differentiation of the precursor cells was clearly demonstrated in tumors of the hemopoietic system, including leukemias and lymphomas. In these tumors detailed relationships between differentiation and oncogenic transformation have been studied most extensively. These relationships are considered below. The characteristics and role of differentiation in carcinomas (epithelial tumors) have now been described.

First, two characteristics of differentiation of carcinomas should be noted: 1) a decrease in differentiation manifested during tumor development; 2) preservation of differentiation potency of the tumor cells even when differentiation markers are apparently lost.

The wide spectrum of differentiation levels of carcinomas and the tendency for decreased differentiation

0006-2979/00/6501-0107$25.00 ©2000 МАИК “Наука/Interperiodica”

are determined by two factors, the differentiation level of the precursor cells and the permanent evolution (progression) of the tumor.

Tumors do not evolve at all stages of differentiation. As a rule, their precursors are stem and commited stem cells at an important stage of differentiation that are determined to develop by several or a single route and that are able to maintain themselves for a limited or unlimited time [6]. These cells are at the "shortest distance" from the stem tumor cells because transformation from limited maintenance to unlimited maintenance (immortality) and from regulated proliferation to autonomous growth is enough for oncogenic transformation.

Transformation occurring during the stage of committed precursor terminates differentiation at this very stage and blocks ontogenesis of the cell line [7], thus lowering the level of differentiation. Indeed, transformation "freezes" the direction and level of differentiation of the precursor cells. Then, tumor progression becomes important, including accumulation of mutations and selection of the clones with maximal proliferation, maximal independence from the controlling host factors, and aggression, i.e., clones which can replace non-homologous tissues or which have invasive and metastatic abilities. Evidently, in tumors the loss of structures and functions performed by normal tissue for the host should correspond to the goal of progression. The pathways of such reduction during tumor evolution are strictly individual [8]. It is poorly understood why this reduction is not absolute; why hepatomas begin to synthesize αFP, which they do not need, and plasmocytomas synthesize Ig, which has no biological activity, whereas small-cell carcinomas synthesize neuroendocrine peptides. Why are non-specific tumors not known? Why does a tumor preserve at least some differentiation markers of the parental tissue [9]?

Manifestation of the tissue-specific differentiation state in tumors not only involves preservation of tissue-specific structures and functions, but also (in a more specific manner) includes preservation of differentiation potencies attributable to the tumor precursors. This was clearly demonstrated for the first time in a model of experimental teratocarcinomas, which are tumors originating from the germinal cells with differentiation potencies characteristic for early embryonic cells [10, 11]. These tumors can be transplanted *in vivo* or maintained *in vitro*, and they have some features of a typical fully malignant tumor without any signs of differentiation; this corresponding to its origin from the non-differentiated germinal cells. In transplanted teratocarcinomas, regions of mature tissues can appear and isolated cells of the tumor can differentiate in several directions, demonstrating the classical properties of the polypotent stem cell [12]. During serial passage *in vitro* without differentiation signs, differentiation of the cultured cells to various tissues can be triggered by retinoic acid [13]. Differentiation potencies of teratocarcinomas were clearly demonstrated in the classical experiments of Mintz and Ilmensee when cells of non-differentiated teratocarcinoma were implanted into a blastocyst of a murine embryo; a chimeric mouse developed which contained normal tissues originating from the embryonic cells and the cells of the teratocarcinoma. Moreover, these mice gave normal offspring with a marker mutation specific for teratocarcinoma [14]. These experiments clearly proved that the tumor preserved its ability to differentiate.

Reversion of lowly differentiated tumors to more or highly differentiated forms was demonstrated in cultured hepatomas [15] and mammary tumors [16]. In both cases, differentiation was induced (transfection with liver-specific transcription factor HNF-4 gene in hepatoma [15] and anti-integrin antibody treatment in mammary carcinoma (MC) [16]). In both cases, the tumors preserved their ability to differentiate in an organ-specific manner. In remains unclear why carcinomas do not lose this ability. Only certain particular reasons are known that require preservation of tissue-specific function and tissue-specific differentiation. These reasons include preservation of hormone dependence in tumors of hormone-dependent tissues (MC [17] or prostate cancer (PC) [18]). In both cases, at least during the early stages of progression, receptors to estrogens (MC) and androgens (PC) are preserved; these receptors are vitally important for survival of the corresponding tumors, but they do not participate in transformation. Hormone receptors can be lost during evolution of these tumors, thus increasing their autonomicity.

Other cases of preservation of initial characteristics of the parental tissue include auto- and paracrine stimulation of tumor growth [19]. However, the problem of the relationships between transformation and differentiation is most completely studied in tumors of the hemopoietic system.

DIFFERENTIATION AND ONCOGENIC PHENOTYPE IN HEMOBLASTOSES

Tumors of the hemopoietic system are a clear example of the preservation of a differentiated state of normal cells during their oncogenic transformation. The most detailed classification of leukemias and lymphomas is based on their immunophenotyping, which reveals preservation of CD-cluster differentiation characterizing a certain direction and stage of differentiation of the hemopoietic system cells [20]. Moreover, certain differentiation antigens (for example, CALLA, that is CD10) were discovered in leukoses and considered as specific tumor antigens; only later was CALLA demonstrated in transient stages of normal differentiation [21]. A combination of differentiation antigens in certain lowly differ-

entiated acute leukemias also corresponded to early stages of differentiation of hemopoietic cells, when a certain program of further development was not yet established and the elements of various lines of hemopoietic differentiation are present [22]. Differentiation state of hemopoietic tumors strictly corresponds to the concept of **oncogenesis as a "frozen" stage of ontogenesis**, i.e., preservation of the direction and stage of differentiation of the precursor cell by the tumor [7, 23]. This "freezing" does not imply similar differentiation of all cells in a population, but rather corresponds to characteristics of the stem tumor cell during its origin from a committed stem hemopoietic cell. The stem cell of leukemia can differentiate to a certain extent, and this differentiation is manifested in variable maturation in a tumor population.

Preservation of a highly differentiated state in hemoblastoses seems to be mysterious and illogical. Why and what for? Tumor progression implies selection for autonomic and uncontrolled by the host proliferation; thus, tumors should lose all excessive elements that are not required for survival and aggression. Oncogenes determining malignancy are predominantly directed towards autonomization of the cell cycle so that it is not controlled by the mechanisms of suppression [24]. A clear example of transformation of hemoblastosis from relatively benign chronic forms to a lowly differentiated *acute* form is blastic crisis during chronic myeloid leukemia (CML). However, in this case as well, the cells of the blastic crisis preserve the phenotype of the immature myeloid, lymphoid or erythroid precursors [25]. Evidently, production of immunoglobulins by plasmacytes does not promote their proliferation or dissemination. However, it is not lost. Hence, preservation of differentiation state can be required for maintenance or appearance of an oncogenic phenotype. Alternatively, a differentiated state is an adverse consequence of oncogene function that is difficult to lose. Under normal conditions, if a protooncogene controls the cell cycle, then its transformation into an autonomic oncogene activates (or maintains) both processes or the state of the cell. The two possibilities do not exclude each other. Considering this, several question are discussed in this section. What is the possible relationship between differentiation mechanism and the appearance and maintenance of oncogenic phenotype in hemoblastoses? How potent are tumors of the hemopoietic system in increase and induction of differentiation, and is it possible to use differentiation potencies of leukoses and lymphomas in their therapy?

RELATIONSHIP BETWEEN DIFFERENTIATION AND ONCOGENIC TRANSFORMATION

Differentiation of a certain cell type can be considered as expression of a complex of the genes specific for this cell line. In turn, expression of these genes is controlled by regulation regions of the genes, including *promoters* and *enhancers*. The promoter is the starting point in gene transcription, and transcription requires RNA-polymerase II and activating factors. Enhancers are the regulatory sites of DNA located at a certain distance from the controlled genes, but in the same locus of the chromosome. Activation of enhancers and regulatory sites of the promoter significantly stimulates the activity of the gene, i.e., accelerates its transcription. Promoters and enhancers are activated by specific binding of tissue-specific nuclear transcription factors (TF); the spectrum and specificity of TF determines the direction and level of differentiation of a certain cell line. To interact with TF, promoters and enhancers of the tissue-specific genes should be opened, i.e., they should not be blocked by chromatin proteins that occupy enhancers and genes inactive in a certain cell type [26].

Thus, differentiation of cell lines involves interaction of certain tissue-specific TF with enhancers and promoters of the tissue-specific genes. This interaction induces the expression of the differentiation genes, which determine the specificity of the cell line. At present, actively studied problems include the formation of certain tissue-specific TF and the regulation of chromatin configuration that enables the interaction of TF with enhancers and promoters and RNA-polymerase II binding to promoters of the genes in various cell types.

Master genes controlling crucial points of hemopoiesis are especially important for understanding the role of differentiation in the genesis of leukemia. Master genes activate the synthesis of large blocks of TF, which finally determine the expression of the cell line-specific genes.

The list of master genes of the hemopoietic system includes *PU1* and *LMO2* which control myeloid differentiation, the *GATA* master gene responsible for erythroid differentiation, and *IKAROS* that is critical for development of the common T and B cell precursor [27-29].

A certain line of myeloid or lymphoid differentiation is characterized by functioning of the specific master genes; for example, *E2A/PAX5/Id* are important for B cell differentiation; a combination of more general master genes is required for the initial stage of T cell differentiation [27, 28]. These genes play a specific role in the occurrence of acute leukemia; their participation in oncogenic transformation is considered below when the corresponding hemoblastoses are discussed.

The relationship between cell transformation and differentiation can be considered as a problem of participation of the differentiation factors in formation or maintenance of the malignant phenotype, i.e., activation of protooncogenes or inactivation of tumor suppressor genes [24].

PROTOONCOGENE ACTIVATION BY TRANSLOCATION IN THE REGION OF THE IMMUNOGLOBULIN (Ig) OR T CELL RECEPTOR (TCR) GENES

Burkitt lymphoma (BL) is a classical system that enabled clear determination of the association between cellular protooncogene activation and the main factors of B cell differentiation. Activation of the cellular protooncogene c-*MYC* and loss of its control by cellular systems is tightly coupled to the B cell differentiation mechanisms. In BL, crucial events in B cell transformation include translocation (*t*) of the c-*MYC* oncogene into the region of the IgH gene (*t* (8; 14))[1], or of the Igκ gene (*t* (2; 8)), or the Igλ gene (*t* (8; 22)) [30-32].

Herpes-like Epstein–Barr virus (EBV) is important for the etiology of African Burkitt lymphoma. Infection of a B cell with EBV induces its proliferation while differentiation in the mature B cell continues. Assembly of variable (*V*) genes of heavy (IgH) and light (IgL) chains of Ig includes genetic recombination of the V_H- and V_L-fragments of the genes. This recombination is started at a pre-B cell stage by specific enzymes, recombinases. Recombination errors result in transfer of a fragment of chromosome 8 to the region of the Ig genes (chromosomes 14, 2, and 22 corresponding to the *H*-, *L*κ-, and *L*λ-genes); the cleaved site of the transferred fragment contains the c-*MYC* gene whose signal sequences are similar to that of the Ig genes[2]. Recombination errors are thus possible, and they result in transfer of the c-*MYC* to the region of the *IgH* or *IgL* gene. The c-*MYC* gene becomes located in the region of activated *IgH*- or *IgL*-enhancers of the B cells and is constitutively expressed.

In this case the translocation is apparently tissue-specific because it involved physiological recombination characteristic for B cells. It should be noted that chromosome 8 containing c-*MYC* is transferred to the regions of chromosomes 14, 2, and 22, which are open in B cells. Thus, three differentiation factors (specific TF, enhancers, and chromatin configuration) are involved in oncogenic transformation in BL. Moreover, the recombination resulting in transformation is also specific for B cell differentiation.

The activity of Ig-enhancers is the main factor not only in B cell transformation, but also in the maintenance of oncogenic phenotype of BL. This phenotype is tightly associated with the differentiated state of the B cells and is coupled to that state. This was demonstrated in endemic African lymphoma that develops concomitant to EBV infection and tropical malaria. Sometimes, in spontaneous BL, transposition of c-*MYC* to the *IgH* locus separates c-*MYC* from the *IgH* enhancer. However, Ig is still actively and tissue-specifically expressed, but this expression does not require the classical Ig-enhancer. The *IgH* expression includes c-*MYC* as well [32].

A similar situation is observed in the case of human follicular B cell lymphoma [31]. In this tumor, the apoptosis-suppressing *BCL*-2 gene [24] is translocated to the *IgH* enhancer-dependent region and becomes permanently activated. *BCL*-2 protects B cells from apoptosis, thus resulting in their immortalization, which is required for transformation [34]. In this case, three differentiation factors are involved, and maintenance of transformed phenotype is tightly associated with the differentiation state of the cell. In follicular lymphoma, tissue-specificity corresponds not only to obligatory activation of the *IgH* enhancer, but also to incorporation of *BCL*-2 in immunogenesis as a specific B cell component, thus providing for a longer life span of the memory B cells [35].

It is noteworthy that *BCL*-2 does not stimulate cell proliferation and transgenic *BCL*-2 mice only have increased risk of follicular lymphoma. To induce follicular lymphoma, additional mutations are required concomitant to B cell hyperplasia caused by *BCL*-2 overexpression [32, 36].

The role of the *IgH* locus in translocation specifically associated with B cell malignancy was shown in many other B cell lymphomas. For example, translocations *t* (14; 19) and *t* (11; 14) are typical for certain lymphomas. This stimulated a search for new protooncogenes in the contact site of chromosomes 19 and 11 with chromosome 14 where functional *IgH* is localized [31, 32].

As a result of this search, the *BCL*-1 and *BCL*-3 protooncogenes were identified. *BCL*-1 is localized in chromosome 11 at the cleavage site of *t* (11; 14). This gene is identical to the *PRAD*-1 (parathyroid adenoma) gene. *PRAD*-1 is one of the regulators of the cell cycle, cyclin D1. Its activation during *t* (11; 14) is characteristic for 50% of lymphomas of the embryonic centers [31, 37].

The *BCL*-3 protooncogene was isolated in the case of *t* (14; 19) in cells of chronic lymphatic leukemia (CLL). This protooncogene is activated during B cell proliferation, and thus it influences the pathogenesis of CLL [31].

The interesting translocation *t* (1; 14) was studied in intestinal mucosa-associated lymphoma. In this case the *BCL*-10 gene stimulating apoptosis in altered (mutant) cells becomes controlled by the *IgH* gene enhancer. The *BCL*-10 gene located in the *IgH* region (where hypermutations are a physiological mechanism of immunogenesis) begins to mutate at increased rate and loses its nor-

[1] Numbers in the parentheses correspond to ascending chromosome numbers.

[2] Signal sequences are short DNA sequences adjacent to the *V* gene fragments (*V*, *D*, *J*), which are joined during differentiation forming a single *VDJ*- or *VJ*-gene. Signal sequences are recognized by recombinases assembling the *V*-gene [33].

mal function. Thus inactivated, *BCL*-10 can provide for survival of mutated cells, promoting the formation of a tumor clone [38].

In mouse plasmacytoma, similar to BL, *c-myc* is translocated to the *IgH* enhancer region [31]. In this case recombination error apparently occurs during the generation of the memory B cells which is associated with the second recombination corresponding to the transfer of the assembled *VDJ* gene to the regions of the genes responsible for the synthesis of secondary response Ig (predominantly IgA). In this case, *c-myc* is located in the same region of Ig-enhancer as in BL, but this region is translocated to the *C*α-gene.

Essentially similar events were described in human T cell lymphoma with translocation *t* (8; 14) in the region of the locus of the α-chain of the T cell antigen receptor (TCR) in chromosome 14 and c-*MYC* in chromosome 8 [37].

Thus, activation of a protooncogene by translocation to the region of tissue-specific *Ig* or *TCR* enhancer is one of the typical and frequent pathways associating oncogenic transformation and cell differentiation. This pathway is characteristic for lymphatic leukemia and lymphomas when genetic recombination is the main factor of differentiation. In this case, classical oncogenes directly induce autonomization of the cell cycle (*MYC*, *PRAD*, *BCL*-3) or immortalization (*BCL*-2).

Activation of transcription factors controlling differentiation is a specific pathway of oncogenic transformation for lymphatic leukoses occurring via translocation to the *Ig* or *TCR* loci. The family of these factors is activated in T cell leukemia by translocations *t* (11; 14), *t* (14; 19), *t* (1; 14), and *t* (10; 14) [31, 32, 39].

It should be emphasized that sometimes TF that function normally in nerve cells or hepatocytes are activated [31].

Involvement of TF **not in a proper place** or **not in a proper time** blocks normal maturation of T cells but does not reverse previous differentiation stages. The family of differentiation genes *LMO* and proteins controlled by this family is studied in great detail [28]. For example, the Lmo2 protein controls the very first stages of hemopoietic differentiation. It is constitutively expressed when the *LMO*2 gene is translocated to the *TCR*β region during recombinant assembly of the *TCR*β gene in T cells from its *V*-, *D*-, and *J*-fragments. Abnormal expression of the Lmo2 protein blocks early stages of T cell differentiation and is one of the steps required for the formation of acute T cell lymphatic leukemia (T-ALL). It should be noted that constitutive expression of *LMO*2 by itself is not enough for oncogenic transformation, and additional mutations are required to induce a malignant T-ALL clone [28].

T-ALL is a rather large group of acute lymphoid hemoblastoses resulting from translocation of the regulatory TF genes to the region of the *TCR* genes [28, 31, 36].

Thus, the group of T cell leukemia uses a mechanism of gene activation resulting in oncogenic transformation. The mechanism includes translocation of the genes to the region of activation of the tissue-specific *TCR* gene. Hence, activation of abnormal TF with tissue-specific differentiation is required for transformation.

CHIMERIC GENES AND FUSED PROTEINS

Another important mechanism of tissue-specific transformation involves the formation of chimeric genes on the borderline of chromosome translocations.

The list of leukoses includes acute pre-B cell leukemia of children with chimeric gene *E2A/PRL* formed by translocation *t* (1; 19) [31, 40]. In this neoplasm, fused E2a/Prl protein substitutes for the normal product of the *E2A* master gene and blocks the exit of the cells from the pre-B cell stage. The *E2A* master gene (see above) controls the synthesis of the transcription factors e47 and e12 which interact with the *Ig*κ gene enhancer and induce transition of the pre-B cell into immature B cell synthesizing the complete molecule of the membrane-bound IgM. Fused E2a/Prl can be associated with the corresponding enhancer, but subsequent events are more complex due to the influence of the Prl-fragment of the fused protein. Oncogenic transformation of the B cell requires a block of differentiation but apparently is not limited to the block. This form of B-ALL must preserve the selected differentiation pathway because the blocking activity of the chimeric gene (a crucial component of pre-B cell transformation) can be manifested only in the framework of this pathway.

An essentially similar situation is observed in B-ALL associated with *t* (17; 19) [41]. The *E2A* gene is localized on chromosome 19, and the *HLF* gene is localized at the chromosome 19 fusion site of chromosome 17; the latter gene encodes for a protein similar to the *trans*-factors specific for liver and kidney. The chimeric *E2A/HLF* gene is activated in B cells and controls the synthesis of the fused E2a/Hlf protein. This protein is an analog of E2a, and it is involved in further development of the pre-B cell; however, inclusion of the Hlf fragment alters and blocks the developmental process.

Considering myeloid leukemias, the chimeric *BCR-ABL* gene is the classical example; this gene is formed by translocation *t* (9:22) (Philadelphia chromosome), and it is an absolute cytogenetic marker of CML [25]. The translocation (depending on exact position) results in the synthesis a variant fused protein, i.e., p210 or p190. The former protein is the marker of CML, and the latter is associated with ALL [31]. The *BCR-ABL* gene is constitutively expressed in CML. The *BCR* portion corresponds to the promoter of the chimeric gene, and the *ABL* portion is the classical protooncogene. In normal cells, Abl is a tyrosine-specific protein kinase involved in the major signal transduction pathways in the cells.

The function of Bcr-Abl p210 is not completely understood, but it was found that its transforming effect in CML is realized only in myeloid differentiation, although Philadelphia chromosome is detected in all lines and stages of hemopoiesis starting from the stem cell [25, 29]. Similar conclusions were reached in experiments on transplantation of bone marrow from mice transfected with the human *BCR-ABL* gene to irradiated mice. The recipient mice developed CML, thus suggesting that *BCR-ABL* induces CML and that the gene has a specific effect in a given myeloid differentiation pathway [42].

In myeloid differentiation CML, Bcr-Abl blocks apoptosis, stimulates autonomous proliferation independently of growth factors, and induces macrophage differentiation [43]. Myeloid cells are thus accumulated in a huge numbers; these cells differentiate but do not undergo apoptosis. Hence, CML is a sort of benign tumor until additional mutations fail to cut off the myeloid line of differentiation from the transformed precursor, and CML becomes an acute blastic crisis.

Thus, CML is tightly associated with the differentiated state of the myeloid line, and evidently the effect of *BCR-ABL* can be realized only within the framework of this line, where this chimeric gene functions and induces simultaneous proliferation and differentiation.

The clearest and most informative system where the transforming activity of a chimeric gene and fused protein has been demonstrated is the system *PML-RAR*α (promyelocytic leukemia/retinoic acid receptor α) that is a consequence of translocation *t* (15; 17) [29, 44, 45]. *PML-RAR*α is an analog of the *RAR*α gene encoding for a normal nuclear receptor (Rar) of retinoic acid (RA), one of the most active and universal inducers of differentiation. Rar is included in the complex of nuclear proteins composed of TF of myelocyte differentiation and histone acetylation and deacetylation enzymes. This complex is a repressor of myeloid differentiation genes. In the promyelocyte stage, RA binds to Rar with high affinity. Formation of Rar-RA inactivates the repressor complex blocking the differentiation genes, thus enabling subsequent differentiation of promyelocytes. Fused Pml-Rar protein is included in the receptor complex but has low affinity for RA and does not bind it at physiological concentration. The block of differentiation is not removed by the repressor complexes containing Pml-Rar. The cell does not advance through differentiation stages but remains a promyelocyte. Acute promyelocytic leukemia occurs in the population of cells containing the chimeric *PML-RAR*α gene. However, two important points remain unclear: how promyelocyte proliferation is induced and how an immortalized line of leukosis originates in the *PLM-RAR*α-positive population of the cells. Similar to CML, the fusion protein alone is not enough to cause clinical symptoms in this system [44].

Beneficial therapeutic effect of high doses of RA compensating for the low affinity of Pml-Rar is an important consequence and clear confirmation of this point of view (see below).

In this system, normal physiological mechanisms of differentiation are the critical factors involved in induction of leukosis and maintenance of its oncogenic phenotype.

This section considers the most studied and typical cases of formation of the chimeric gene resulting in lymphoid or myeloid hemoblastoses. A complete list of hemoblastoses resulting from chromosomal translocations can be found in several reviews [29, 31, 40, 46]. In these tumors, 45% ALL and 45% AML are associated with translocations specific for a certain form [40].

A general principle indicates that activation or inactivation of the master gene (which is usually involved in the earliest stages of cell line differentiation) induces acute leukemia, whereas activation of the gene controlling proliferation or apoptosis usually results in chronic hemoblastosis [46].

The list of pathways of oncogenic transformation involving differentiation mechanisms is not limited to protooncogene translocation to the region controlled by the tissue-specific enhancer, mutation of the protooncogene that is the differentiation TF, and formation of chimeric genes.

Induction of an autocrine loop by oncogenic viruses, which provide for autonomous growth of the tumor in the host organism, is another pathway using differentiation state. This pathway has been demonstrated in human T cell leukemia associated with HTLV-I virus [47]. Viral gene *pX* controls the synthesis of the p40 protein, which activates T cells, stimulates production of IL-2 (interleukin-2, T cell growth factor), and simultaneously induces IL-2 receptor. Both components, receptor and IL-2, are specific products of T cell differentiation. Stimulation of the T cell by its own growth factor results in its proliferation and escape from apoptosis. Autocrine growth is the initial stage of appearance and progression of T cell leukemia. This mechanism apparently also involves the differentiated state of the T cell and is tightly associated with this state.

Preservation of the differentiated state in all forms of hemoblastoses (see beginning of this section) indicates that there may be other forms of interaction between transformation and differentiation including, for example, paracrine regulation based on tissue-specific mechanisms. The same is suggested by cellular specificity in the occurrence and manifestation of biological effect in numerous translocations inducing tumors [48].

INDUCTION OF DIFFERENTIATION IN LEUKEMIC CELLS. THERAPEUTIC APPLICATIONS

Some classical cell lines grown *in vitro* have hemopoietic origin. For example, the cell line K-562 origi-

nates from a patient with CML during blastic crisis with elements of erythroblastoid differentiation. This cell line preserves low levels of erythroid differentiation markers.

The lowly differentiated cell lines HL-60 and U-937 originate from promyelocytic and monocytic leukemia. These cell lines have been cultured for many years and do not show any clear signs of differentiation, but nevertheless they preserve the ability to advance to certain mature forms.

For example, K-562 cells induced by dimethylsulfoxide (DMSO) differentiate in the erythroid direction to the reticulocyte stage synthesizing hemoglobin and glycophorin, which are the markers of advanced erythroid maturation [49, 50]. DMSO is a low-molecular-weight compound widely used for induction of differentiation in various cell lines of hemopoietic or epithelial origin. Retinoic acid (RA) and promotor of carcinogenesis phorbol ester (TPA) have similar effects, inducing differentiation of various cell lines.

RA, TPA, and tumor necrosis factor (TNF) induce macrophage maturation of cell lines HL-60 [49, 51, 52] and U-937 [44, 51]; during this, the cells change their morphology and, similarly to macrophages, adhere to plastic and express typical macrophage markers [49, 51, 52].

B cell hemoblastoses can also advance through differentiation stages sometimes reaching the levels of the mast cells. For example, the cells of macroglobulinemia can spontaneously differentiate *in vitro*, forming mast cells [53]. The cells from 25 patients with non-Hodgkin lymphomas increased their differentiation level up to Ig secretion when treated with RA or interferon α (Int-α) or their combination [54]. Very clear data were obtained in experiments on the interaction of mouse B lymphomas with the T cells *in vivo*, resulting in induction of Ig-secreting plasmocytomas [55].

Classical studies of Metcalf [56, 57] were especially important for understanding the differentiation of hemopoietic cells and primarily myeloid lines; these experiments identified a number of growth factors influencing critical stages in various lines of myelopoiesis [58]. Terminal differentiation of the cells of myeloid leukoses *in vitro* was shown to be induced by growth factors of myelopoiesis [59]. At the same time as Metcalf studies, Sachs did a series of studies on the induction of differentiation in human and animal leukemias (predominantly myeloid) by natural growth factors [60, 61]. A possible significant increase in differentiation of myeloleukemic cells was shown up to the mature terminal forms *in vivo* and *in vitro*.

These studies had a great impact on the development of therapy of leukoses by induction of terminal differentiation, and recent advances have resulted in major progress.

The most impressive examples of differentiation therapy include treatment of hairy-cell leukosis (HCL) with Int-α, treatment of promyelocytic leukosis (PML) with RA, and (in part) treatment of CML with α-interferon.

In the first case, prolonged remission of HCL (B cell leukosis) was observed during long-term Int-α (a potent regulatory cytokine) therapy [62-64]. Int-α is a protein produced by leukocytes; it has potent anti-viral activity and stimulates activation and differentiation of several types of immune system cells. The mechanism of the therapeutic effect of Int-α on HCL is poorly understood. Probably, Int-α therapy increases the level of HCL differentiation, advancing it from the stage sensitive to growth factors up to the more mature state, which is not sensitive to these factors [62, 64].

RA has an impressive effect on PML (as discussed above). RA receptor (Rarα) is a nuclear protein included in the repressor complex with the differentiation TF. In PML cells, the chimeric *PML-RAR*α gene is formed, and this gene encodes for the Pml-Rar protein with low affinity for RA; this protein does not function at physiological concentrations of RA. A large excess of RA restores the function of the fused Pml-Rar protein, thus removing the differentiation block in promyelocytes. PML differentiates into the terminal forms, and the clinical effect is highly beneficial [44, 45].

Administration of Int-α in CML is not that effective, but in some cases clear clinical benefit is achieved, especially if the therapy is started during the early stages of the disease, is continued for a long time, and is given in combination with other treatments [25]. Disappearance of a Philadelphia chromosome-containing clone is most important in therapy of CML. In this case, Int-α can have a normalizing (differentiating?) effect on an abnormal cell clone and on the hemopoietic system suppressed by the tumor clone. Complete remission is rare, and recurrent cases contain Philadelphia chromosome again.

There is a new differentiation approach that is very promising. This approach uses the specific influences on the differentiation receptor of myeloid precursors, CD44, which interacts with hyaluronic acid; this is why these cells require a specific microenvironment. *In vitro* influence on CD44 with monoclonal antibodies against it or hyaluronane (active fragment of hyaluronic acid) induces differentiation in all forms of acute myeloid leukemia [65]. This study opens a broad perspective for universal differentiation therapy of acute myeloid leukemia.

Thus, treatment of hemoblastoses by induction of differentiation is very important and promising.

The facts and considerations presented herein indicate that preservation of differentiated state of the precursor cell in hemoblastoses is not accidental. Oncogenic transformation of hemopoietic cells, at least in a number of systems, involves mechanisms of normal differentiation and can be considered to rely upon these mecha-

nisms. They are involved in transformation (physiological recombination errors) and maintenance of tumor phenotype (protooncogene activation by enhancers, maturation block, induction of differentiation by fused protein). Tight association between transformation and differentiation can be a unique property of hemoblastoses, thus enabling their therapy via induction of differentiation.

POSSIBLE REASONS FOR DIFFERENCES IN DIFFERENTIATION STATE BETWEEN HEMOBLASTOSES AND CARCINOMAS

Preservation of differentiation signs and potencies are attributable to carcinomas as well as hemoblastoses. In the case of hemoblastoses, the preservation appears as "freezing" at the stage of oncogenic transformation of the precursor cell, and in the tumor this cell is maintained in the very same differentiation state. In this case, transformation does not require a decreased level of differentiation and is not only fully compatible with differentiation, but also uses its mechanisms. Intercellular interactions are not important for transformation and progression of hemoblastoses. The cells of these tumors are constantly moving and they only transiently contact with stroma, endothelium, or with each other, exchanging information with the help of cytokines.

In carcinomas the situation is completely different. Transformation may or may not involve differentiation mechanisms [24], but progression from the earliest stages of cancer requires alterations in intercellular interactions tightly associated with differentiation. One of the basic concepts in fundamental oncology is the distinction between *initiation* and *promotion* during tumor development [26]. Initiation can be considered as an oncogenic mutation or transformation, but promotion is *required* for *manifestation* of the transformed cell or microclone so that it can become an evident and growing tumor. Chemical or physical carcinogens with mutagenic properties are the initiators; physiologically active compounds that do not induce transformation but promote manifestation of the transformed cells are the promotors. Promotors include crotonic oil, phorbol esters, and, probably, estrogens and androgens. Weinberg [66] suggested that surrounding normal tissue inhibits the growth of the tumor cells trying to normalize them and suppressing uncontrolled growth. The normalizing factors (according to Weinberg) can include interaction of the cells with extracellular matrix (ECM), intercellular gap junctional contacts, and cytokines secreted by the normal cells. A normal microenvironment is the first barrier that should be overcome by the transformed clone before it can become an actively growing tumor.

However, at the same time, all these factors are the main factors of microenvironment that induce and maintain differentiation of epithelial cells. ECM is essentially the main factor required for creation and maintenance of epithelial differentiation [67]; gap junctional contacts unite epithelial cells in a tissue and cytokine (for example, tumor growth factor β, TGFβ) suppress cell proliferation. Certain physiological activities of the promoters block intercellular interactions, thus releasing the tumor clone from the control of the normal microenvironment [66, 68].

Thus, to begin the growth, a tumor clone should change its contacts with the surrounding cells so that it is insensitive to their influence. This can result from changes in the microenvironment due to the activity of promoters or to alterations in the transformed cells that are not directly related to transformation. Such alterations can involve the integrin system responsible for cell contacts with ECM [67], and this can be a universal mechanism explaining how the transformed epithelial cell becomes insensitive to its microenvironment.

Bissel et al. [16, 69, 70] have studied this situation in great detail in human mammary carcinoma (MC). It was demonstrated that transformed lowly malignant line of MC is immortal but highly differentiated; it has normal contacts with ECM and forms non-proliferating gland structures in the three-dimensional (3D) matrix. Mutation of this cell line resulting in a clone hyperexpressing the β_4/α_6 integrin complex lowered the level of tumor differentiation and induced chaotic growth of the cells that did not form organ-specific secreting islets [16]. Treatment of the mutant cell line with monoclonal antibodies against β_4-integrin decreased the level of β_4/α_6 in the mutant cells and restored its organ-specific growth in 3D-ECM, lowering its tumorigenicity [16, 69]. It was suggested that in this case ECM interacted with the integrin system and influenced the structure of the nuclear matrix, which in turn activated transcription of the tissue-specific genes of the mammary gland [70].

Anyhow, in this case the system was influenced by factors determining tumor progression that were not directly associated with transformation but were coupled to tumor differentiation. Decrease in differentiation level of the tumor was not due to the initial transformation of the MC precursor cell but resulted from the required stage of the tumor clone progression.

The classical system of multistage carcinogenesis of intestinal tumors is another example [71]. One of the genes of this tumor, *SMAD*4, is associated with the tumor growth suppressor *DCC*; it blocks signal transduction from the TGFβ receptor that controls the c-*MYC* protooncogene and suppresses cell proliferation. Mutation of *SMAD*4 in the initiated clone of polypus removes the suppressive effects of the microenvironment (TGFβ), promoting the subsequent stage of progression [72]. This case is also an example of separate genetic control of initiation and progression as well as of decreased differentiation of the tumor due to disappearance of the influences of the microenvironment.

A critical role of ECM in the control of αFP expression in hepatocytes was demonstrated [4]; αFP is one of the most demonstrative embryo-specific tumor markers. In these cells, αFP synthesis *in vivo* is determined by hepatocyte inclusion in the hepatic plate [73], and synthesis *in vitro* depends on association with 3D-ECM but not on adherence to 2D-matrix of the same composition [74]. In the liver plate and 3D-ECM, αFP synthesis is suppressed. A mixed culture of hepatocytes and epithelial non-parenchymal cells of the liver (IAR) maintained differentiation of hepatocytes and completely inhibited the αFP synthesis. The *ras* gene-transformed IAR form incomplete matrix in the mixed culture, and this results in dedifferentiation of hepatocytes and in αFP synthesis by these cells [75].

These experiments suggest that reexpression of αFP by the hepatic tumors is an example of an *epiphenomenon*, i.e., a result of loss of sensitivity of the transformed hepatocytes to the influence of the normal microenvironment, primarily ECM [76]. Integrins can be important in this system.

Another example of loss of control of the regulatory systems over the tumor with apparent lack of tissue specificity is the loss of hormonal dependence by prostate cancer. In this case, it was demonstrated that the signal induced by androgen binding to the hormone receptor begins to be generated by a different receptor Her2/neu or an intracellular protein enhancing the effect of minimal amounts of the hormone–receptor complex [77]. Hyperproduction of this signal-enhancing factor results in growth independent from physiological androgen concentration, thus providing for maintenance of the tumor when the specific receptor level is significantly decreased. Hence, tumor the differentiation level is decreased but the differentiation mechanisms are preserved. Thus, in carcinomas transformation can "freeze" the cell at an early or advanced differentiation stage, but progression required for tumor manifestation makes it insensitive to the influences of the system of intercellular interaction, thus inevitably decreasing the level of differentiation; the differentiation potencies are often (or as a rule) preserved. This determines similarities and differences of carcinomas versus hemoblastoses. Connective tissue tumors (sarcomas) are studied to a lesser extent with respect to this phenomenon.

This work was supported by the Leading Scientific School grant No. 96-15-98082.

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