ONCOLOGY

Molecular Markers of Tumors

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 8, pp. 199-208, August, 2009
Original article submitted April 29, 2009

The main biologically significant molecular markers of human tumors are discussed on the basis of modern published data and author's findings of many-year studies: steroid hormone receptors, growth factors and underlying signal proteins, tumor-associated proteases, and angiogenesis markers. Methodological aspects, progress in preclinical studies, international recommendations on the use of these parameters for prediction of the disease course and prescription of effective therapy are analyzed. Latest data on the potentialities and limitations of modern highly productive technologies (microchips) as an alternative to studies of individual molecular markers are presented.

Key Words: *molecular markers of tumors; steroid hormone receptors; growth factors; growth factor receptors; proteinkinases; tumor-associated proteases; angiogenesis markers; microchips*

Molecular markers, also called cellular, tissue, or biological markers characterize individual features of a tumor, its specific "biological behavior" and regulation. The number of parameters tried in clinics and laboratories as potential molecular markers increases fulminantly reflecting the progress and findings in research of the mechanisms of manifestation of the basic characteristics of tumor cells, such as invasion, metastasizing, unlimited proliferation, capacity to resist apoptosis and stimulate angiogenesis, and their sensitivity to exogenous and endogenous regulators.

The parameters already used as actual or potential molecular markers of various tumors are presented in Table 1. They include oncogenes and protooncogenes, oncoproteins, growth factors, their receptors and the underlying signal proteins (all of them are most often products of different oncogenes), steroid and peptide hormone receptors, suppressor genes and products of their expression, hormone-dependent proteins, tumor-associated proteases involved in metastasizing, invasion, and angiogenesis processes, and proteins responsible for cell-cell contacts.

Changes in the number of copies of a gene or its mutation, or changes in the level of gene expression (mRNA) and quantity of synthesized protein or its functional activity, measured under stringently controlled conditions, can be methodologically regarded as molecular markers. Therefore, the entire armory of methods of modern molecular biology, biochemistry, and immunochemistry can be used for detection and measurements of molecular markers.

In clinical practice, evaluation of any molecular marker can lead to two practical results: detection of a subgroup at a high risk of relapses and/or metastases, requiring urgent adjuvant therapy and/or more thorough observation among the patients with early stages, not subjected to adjuvant therapy for other clinical or laboratory parameters; evaluation of tumor sensitivity to certain therapies and prescription of individual protocols of adjuvant therapy to patients with disseminated

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0007-4888/09/1482-0230

TABLE 1. Main Groups of Biologically Significant Parameters Actual and Prospective Molecular Markers of Tumors

Biological significance	Markers
Hormonal sensitivity indicators	Steroid hormone receptors: estrogen receptors (ER), progesterone receptors (PR), androgen receptors (AR), glucocorticoid receptors (GR)
Indicators of auto/paracrine proliferation regulation activity	Growth factors and their receptors: c-erbB family receptors (HER): EGFR and its ligands (EGF, -transforming growth factor, amphiregulin, *etc.*), HER-2/neu; insulin-like growth factor receptors; somatostatin receptors
	Enzymes and proteins involved in mitogenic signal transmission: receptor tyrosine kinases, MAP kinases, PI3K, Akt, NFkB, STAT, Grb2, *etc.*
Metastatic and invasive activity parameters	Plasminogen activation system components: uPA, PAI-1, uPA receptor, PAI-2, tPA
	Matrix metalloproteinases (MMP) and their tissue inhibitors (MPTI)
	Other proteolytic enzymes
	Integrines, cadherines
Neoangiogenesis activity parameters	VEGF A and its types 1 and 2 receptors
	VEGF C and its receptor
	Other angiogenic factors: fibroblast growth factor, thymidine phosphorylase, TNF, IL, *etc.*
Apoptosis regulators	Suppressor genes and their products: *p53*, retinoblastoma gene
	Pro- and antiapoptotic factors: Fas receptor and Fas ligand, bcl-2, PI3K, Akt, NFkB, *etc.*
	Caspases

process. The development of new drugs with effects directed towards these molecules and blocking the processes regulated by these molecules is now becoming one of important practical results of studies of the molecular biological characteristics of tumors.

Since the number of markers, for which potential practical significance was demonstrated in clinical and laboratory studies, is really great, while the price and difficulties of their evaluation are very high, it is important to clearly define the criteria, by which it will be possible to recommend this or that parameter for practical use [26]. The basic conditions for including a marker into patient examination protocol and for practical use of the results of its evaluation are as follows: 1) evaluation of a marker should lead to more favorable results of treatment (prolongation of relapse-free or overall survival and/or improvement of the quality of life, and/or to reduction of the price of treatment; 2) these advantages are to be conclusive: the first-level evidence is to be obtained in a special wide-scale randomized prospective study or as a result of meta-analysis of a significant number of studies carried out on a limited volume of material; 3) available standard reproducible methods with clearly defined criteria and internal and external quality control programs. Very few markers meet these requirements, and hence, the number of parameters actually used and recommended by international organizations for examinations of cancer patients is limited.

For many years preclinical studies of many markers belonging to various biologically significant subgroups [8] were carried out at Laboratory of Clinical Biochemistry of N. N. Blokhin Cancer Research Center.

The presence of steroid hormone receptors is the criterion of sensitivity to endocrine therapy. The first molecular markers, used in practical oncology, are steroid hormone receptors. These proteins specifically and selectively bind the corresponding steroids after their penetration into the cell and mediate their biological effects. Detection of steroid hormone receptors was used for about 40 years for evaluation of hormone sensitivity of breast cancer, prostatic cancer, and endometrial cancer. Due to this, the efficiency of endocrine therapy in receptor-positive patients is improved significantly, while the resistant patients with receptor-negative tumors are not subjected to unnecessary traumatic treatment (ovariectomy) or drug therapy with side effects.

In addition to studies of traditionally hormone-dependent tumors, we carried out screening (by radioligand method) of the receptor status of a variety of malignant tumors: osteosarcomas, melanoma, ovarian cancer, lung cancer, laryngeal, gastric, colorectal, renal cancer, leukemic cells and lymphosarcomas for evaluating the probability of adding hormone therapy to the treatment protocols [22]. Tumors containing receptors to this or that class of steroid hormones

(estrogens, progestins, androgens, or glucocorticoids) were detected in tumors of virtually any localization, and in many cases (osteogenic sarcoma, ovarian cancer, lymphoid leukemia) the prognostic significance of the receptor status for relapse-free and/or overall survival of patients was demonstrated. However, the experience gained all over the world indicates poor efficiency of hormone therapy in these tumors. Only in breast cancer testing for estrogen receptors (ER) and progesterone receptors (PR) is obligatory for all primary patients according to the international recommendations. The need in evaluation of the receptor status of breast cancer for prescription of endocrine therapy was confirmed by meta-analysis, including a total of 37,000 patients who took part in 55 randomized studies [27].

Growth factors, their receptors, and underlying signal proteins are indicators of tumor capacity to autonomous growth. The sensitivity to endocrine stimuli is as a rule intrinsic to well-differentiated tumors, depending to a certain measure on the whole-body regulatory effects or on the external stimuli. However, one of the basic capacities of highly malignant tumors is the capacity to unlimited autonomous growth. This capacity is determined by the effects of growth factors, proteins or polypeptides produced by tumor cells or other components of the tumor tissue (fibroblasts, macrophages and lymphocytes infiltrating the tumor, endotheliocytes) and reacting with specific receptors on the surface of producer cells or adjacent cells, stimulating (as a result of the subsequent complex chain of events) cell division.

The best studied and clinically realized is the mechanism of action of the epidermal growth factor receptor (EGFR) system and the related receptors of the c-erbB or HER (human epidermal growth factor receptor) family. This family includes four proteins: EGFR proper (erbB-1, HER-1) and ErbB-2 (HER-2/neu), ErbB-3 (HER-3), and ErbB-4 (HER-4) (transmembrane receptors of similar structure, whose intracellular part possesses tyrosine kinase activity). The best known ligands of c-erbB family receptors are epidermal and α-transforming growth factors, amphiregulin, and cripto, reacting only with EGFR, and heregulins (neuregulins), reacting with ErbB-3 and ErbB-4 [45]. No ligands reacting with ErbB-2 (HER-2/neu) receptor were found until present.

Many studies, including those carried out at our laboratory, showed that the presence of classical EGFR (HER-1) in the mammary tumor, particularly in the absence of steroid hormone receptors, predicts an unfavorable course of the disease even at the early stages and indicates resistance of endocrine therapy [1,9,18]. However, because of ambiguity of the results, EGFR test did not become a routine clinical practice as a marker of common prognosis or hormone sensitivity of breast cancer.

In addition to breast cancer, EGFR was detected in tumors of different localizations. A series of our radioligand studies with ^{125}I-labeled EGF with subsequent separation on hydroxylapatite (the «golden standard» for EGFR detection in the membrane fraction of tissues) showed the presence of these receptors in 32-75% of malignant tumors (Table 2). They were most often detected in non-small-cell lung cancer (NSLC), which was also characterized by a significant decrease in relapse-free and overall survival at EGFR level >20 fmol/mg protein [16]. These data acquired new practical significance recently, when drugs specifically blocking activity of EGFR were allowed for clinical trials and practical use: monoclonal antibodies to the receptor (Erbitux) and inhibitors of its internal tyrosine kinase (Iressa, Tarceva), realizing the first stage of the mitogenic signal transmission [8,36]. These drugs are already recommended for the treatment of NSLC [38] and tumors of the head and neck, colorectal and pancreatic cancer [8]. On the other hand, the most recent studies showed that not all receptor-positive tumors are sensitive to anti-EGFR drugs (for example, to tyrosine kinase inhibitors), but only those with the *L858R* deletion mutation in the tyrosine kinase domain of EGFR gene (about 5% tobacco-smoking and 40% non-smoking EGFR-positive patients with NSLC) [51]. It is therefore essential to test the presence of this mutation in EGFR-positive patients with NSLC as an obligatory additional criterion of sensitivity to EGFR inhibitors [57]. On the other hand, the efficiency of Erbitux in colorectal cancer is confined to tumors with specific mutations in the *KRAS* gene [55], because only tumors with the "wild" type of this gene are sensitive to the drug.

Creation of Herceptin led to a tremendous breakthrough in the practical use of markers involved in

TABLE 2. Incidence of Epidermal Growth Factor Receptors in Membrane Fraction of Human Malignant Tumors

Tumor location	Number of examined patients	EGFR-positive tumors, %
Breast cancer	291	38 [1,7,18]
Primary tumors of the bones	115	58 [17]
Endometrial cancer	58	32 [2]
Lung cancer	63	75 [16]
Ovarian cancer	52	54 [6]
Esophageal cancer	19	63 [6]
Total	598	47

EGFR-dependent regulation of tumor growth. This drug, humanized monoclonal antibodies to type 2 receptor (HER-2/neu), is a unique representative of the family. It has no ligand of its own and does not react with any of the known growth factors, activating the related receptors, but is nonetheless the key factor in mitogenic signal transmission for all EGF-like peptides and is essential for effective functioning of the entire system [25]. The key role of HER-2/neu is explained by the fact that the basic feature of all tyrosine kinase transmembrane receptors is obligatory dimerization for the realization of kinase activity and subsequent biological effects. Importantly that after binding activating ligand the ErbB family receptors can form homo- and heterodimers; the most active are heterostructures with HER-2/neu participation.

Blockade of HER-2/neu can essentially inhibit or arrest the growth of tumors depending on such stimuli, but effective use of bioactive preparations implies preliminary evaluation of individual sensitivity of the patients to this treatment [47]. A common method for evaluating the sensitivity to Herceptin is immunohistochemical staining of tumor tissues to HER-2/neu protein with subsequent evaluation of *c-erbB-2* gene amplification by fluorescent *in situ* hybridization (FISH) in dubious cases, when the results of immunohistochemical evaluation are not strictly positive or strictly negative. This approach proved to be effective in the treatment of patients with breast cancer, providing maximum efficiency of Herceptin and ruling out the unnecessary expenditures.

As for the prognostic significance of hyperexpression or amplification of *c-erbB-2* gene, despite a vast scope of material (by the present time several tens of thousands of patients with breast cancer were examined in different laboratories all over the world), no universal opinion on the prognostic value of this marker was formed until recently [27]. However, it was recommended starting from 2005 to use the expression (better, amplification) of HER-2/neu together with other factors for the formation of risk groups from patients with early stages [35]. It was also noted that tumors with amplified HER-2/neu gene poorly react to endocrine therapy, but are sensitive to subsequent chemotherapy, and it is assumed that patients with HER-2/neu-positive tumors should be recommended more intense chemotherapy protocols.

Since HER-2/neu has an intra- and an extracellular compartments, degradation of the receptor molecule during dimerization and migration of its external domain into extracellular space are possible. This feature prompted the development of enzyme immunoassay systems for detection of soluble HER-2/neu in the serum or plasma. Published results indicate good prospects of this test primarily for monitoring Herceptin treatment efficiency [42]. However, it is still essential to accumulate more data and define clear-cut quantitative criteria for practical use of serum values.

Our study comprising 59 primary patients with breast cancer (stages I-III) showed that serum level of soluble HER-2/neu in patients with tumors, characterized by high expression of this protein (2+/3+ according to immunohistochemical staining results) were significantly higher than in patients with low expression of HER-2, and after removal of the primary tumor the serum level of HER-2/neu decreased in the majority of patients [21]. These preliminary data indicate that serum values to a certain measure reflect the level of HER-2/neu in the primary tumor and their measurements can become a sufficiently adequate noninvasive method for monitoring the HER-2/neu status during the pre- and postoperative period.

Numerous clinical trials of Herceptin in various protocols for the treatment of breast cancer demonstrated its high efficiency in patients with high expression of the protein and/or amplification of *HER-2/neu* gene in the tumor [24,27]. However, some patients are initially resistant to this drug despite the positive HER-2/neu status of the tumor. In addition, resistance to Herceptin develops often and rapidly (within 1 year) during therapy [8]. All this stimulates the interest to studies of this drug mechanism of action and search for possible causes of the initial and acquired resistance.

Signal transduction process can be schematically presented for the majority of growth factors as successive phosphorylation or dephosphorylation of a series of transmembrane and intracellular proteins, many of which possess enzyme activities of their own. The signal propagates by successive modulation of one protein in the chain by the other. The most important intracellular systems involved in the realization of the effects of various growth factors are the signal pathway including phosphatidylinositol-3-kinase (PI3K) and its underlying effector Akt serine/threonine proteinkinase; the Ras-Raf signal cascade including the system of mitogen-activated proteinkinases.

Representative and comprehensive studies indicate that constitutive activation of Akt (proteinkinase B) and PI3K/Akt signal pathway in general is a key mechanism of HER-2-positive cell resistance to Herceptin and other drugs against EGFR family; in addition, it can be regarded as a mechanism of hormone resistance of receptor-positive tumors [8,33]. In this connection we carried out two studies. In one we evaluated the expression of PI3K p85 regulatory subunit by immunoblotting [34]. In the other the expression of activated (Ser473 phosphorylated) Akt1 [23,32] in tumors and adjacent histologically intact tissues of patients with breast cancer was evaluated by enzyme

immunoassay. It was found that activities of these two components of the same signal cascade changed differently in malignant degeneration of mammary cells. The expression of PI3K was enhanced in tumors of 79% patients and did not depend on the main clinical morphological factors, including the steroid hormone receptor status. Akt1 was activated in only 49% tumors; the incidence of its activation positively correlated with tumor size and malignancy, was higher in ER-positive mammary tumors than in ER-negative ones, and the correlation with the PR status was opposite. These results suggested that despite the cooperative role of PI3K and Akt in the regulation of cell growth and survival, their effects on the clinical course and hormone or drug resistance of mammary tumors can be quite different [33].

Interestingly, studies of tumors and homologous tissues of NSLC patients by the Western blot method showed higher expression of PI3K in the tumors than in homologous tissues of only 5 of 29 patients, the same expression of PI3K in the tumor and homologous tissue in 20 patients, and a lower expression in the tumors in 4 patients [11]. It seems that activation of PI3K in tumor transformation is not organ- or tissue-specific, despite the important role of the corresponding signal pathway in the cell growth regulation and survival processes and a pronounced oncogenic potential of the PI3K proper.

Tumor-associated proteases are indicators of metastatic and invasive potential. The capacity to metastasizing and invasion is the basic characteristic of malignant tumors. The most important mechanism of these processes is destruction of the adjacent basal membrane and extracellular matrix by tumor-associated proteases. One of the key events in these processes is the proteolytic cascade of plasminogen activation in tumor tissue [28], while urokinase plasminogen activator (uPA) plays the main role in the multi-staged chain of proteases, leading to destruction of extracellular matrix. The uPA receptor, located on cell surface, also plays an important role, as the capacity of uPA to activate plasminogen increases after its binding to the receptor. On the whole, plasmin formation process is a cyclic amplification, regulated by the feedback mechanism. In addition to uPA, tissue plasminogen activator (tPA) is involved in this process; its role in the tumors is the opposite: destruction of tumor cells and protection of the adjacent tissues. Activities of uPA and tPA are inhibited by two protein inhibitors, belonging to the serpin family: PAI-1 and PAI-2. It is assumed that they play different roles in tumor growth: PAI-1 protects tumor cells from self-destruction, while PAI-2 inhibits proteolytic processes in the extracellular matrix. Different components of plasminogen activation system can be located on the tumor cells proper and on stromal fibroblasts, lymphocytes and macrophages, infiltrating the tumor, endothelial cells, and hence, we can say that plasminogen activation is mainly a paracrine process. The level and proportion of plasminogen activation system components in tumor tissue can serve as indicators of metastatic and invasive activity of the tumor, and hence, they are biologically significant prognostic factors [10,28].

High prognostic significance of uPA and PAI-1 in breast cancer was demonstrated in numerous representative studies. Multifactorial analysis indicates that they are independent prognostic factors. The first-level conclusive base is already available: the results of a prospective randomized cooperative study, carried out in about 600 patients with early stages of breast cancer [38], and united multifactorial analysis of the findings of 18 research groups, including a total of 8377 patients [41]. These studies showed that high levels of uPA and PAI-1 are independent factors of unfavorable prognosis, more significant than tumor size, malignancy degree, and receptor status and the patient's age. Hence, evaluation of these characteristics in patients with early stages of breast cancer can already be recommended for detection of subgroups at a high risk of relapses and metastases, requiring more intense therapy and monitoring. However, a cooperative multi-center study NNBC 3-Europe is in progress now [46]. It is planned to involve about 6000 patients, and by its results the final conclusion on the efficiency of including uPA and/or PAI-1 in the protocol of obligatory examinations of primary patients with breast cancer without metastases in the lymph nodes will be made.

Being biologically significant prognostic factors in various tumors, the components of plasminogen activation system can become the targets of molecular-directed antimetastatic drugs. In order to evaluate the prospects of their clinical use, it is important to detect the nosological entities of tumors and the groups of patients in whom the best effect can be expected. Based on these, we carried out a wide-scale study of the role of uPA, PAI-1, and tPA in tumors of different location in more than 900 patients. Its main results indicate that a significant coordinated elevation of uPA and PAI-1 content is a virtually universal characteristic of malignant tumors, while the relationship between the expression of plasminogen activation system components and clinical morphological characteristics and prognosis depends on the tumor type [10]. It was therefore suggested that uPA could be a prospective and selective target for antimetastatic therapy in many malignant tumors.

The development of drugs aimed against uPA or other components of plasminogen activation system remains just a prospective trend of research in molecu-

lar target therapy. Proteases (namely, matrix metalloproteases, MMP), whose activation is the final step in destruction of extracellular matrix initiated by the plasminogen cascade, already became the targets of drugs which are now at different phases of clinical trials (Marimastate, Neovastate, Col-3, BMS-275291, *etc.*). These agents are now regarded as not only antimetastatic, but also as antiangiogenic drugs.

We demonstrated in a study of the spectrum of expression of various MMP and their tissue inhibitors (MPTI) in tumors of patients with colorectal cancer, which is still in progress now, that these tumors are characterized by significant elevation of MMP-2, 3, 7, 9, and 13, and of MPTI-1 in comparison with histologically unchanged tissues [4,5,31]. Hence, the use of MMP inhibitors in colorectal cancer can be perspective, bearing in mind that the greatest progress in therapy of this disease was attained by using direct antiangiogenic drugs (Avastin).

Vascular endothelial growth factor is an indicator of neoangiogenesis activity. Growth factors stimulating the development of new vessels in the tumor (neoangiogenesis) play an important role in the regulation of tumor progress [19,30]. Studies of the recent 15 years showed that vascular endothelial growth factor (VEGF/VEGF-A/VPF) is the key regulator of neoangiogenesis [30]. It is a protein inducing active growth of endothelial cells and formation of new capillaries. Many facts indicate that VEGF is characterized by not only proangiogenic activity, but can also be directly involved in the regulation of tumor cell proliferation. This is demonstrated by involvement of VEGF in regulation of survival and proliferation of some tumor cells cultured *in vitro* [54].

The biological effect of VEGF is mediated through its receptors 1 (VEGFR-1/Flt-1) and 2 (VEGFR-2/Flk-1/KDR), typical transmembrane receptor tyrosine kinases [30]. It is known that VEGF is involved in the regulation of many intracellular signal pathways, mitogenic and antiapoptotic. For example, the underlying effectors of VEGF are apoptosis-regulating proteins: Akt (proteinkinase B) and Bcl-2 [30,54].

Representative clinical studies showed that expression of VEGF in breast cancer is essential for disease prognosis and for tumor sensitivity to hormone and rug therapy [19]. Its high level indicates poor prognosis in early and disseminated breast cancer. The role of VEGF signal pathways in cells of breast cancer proper was actively discussed. Some authors think that the level of VEGF and its effectors (VEGFR-1, VEGFR-2, PI3K, Akt, mTOR, *etc.*) can be significant for predicting the survival and sensitivity of mammary tumor to various therapies. Moreover, some components of VEGF signal pathway are now regarded as prospective targets for antitumor therapy. The possibility of tumor growth suppression by antibodies to VEGF (Avastin) and VEGFR-1 and VEGFR-2 specific tyrosine kinase inhibitors (for example, Sutent) is now actively investigated.

We measured VEGF, VEGFR-1, and VEGFR-2 in the tumors and homologous tissues of 46 patients with breast cancer [32]. The levels of VEGF and its receptors were significantly higher than in the adjacent histologically unchanged mammary gland tissues in 73-85% tumors. Tumor capacity to high production of VEGF was detected from the early stages of the disease. A relationship between the VEGF signal pathway components and the hormone dependent parameters was demonstrated: the levels of VEGF and VEGFR-2 increased in PR-negative tissues, the content of VEGFR-2 correlating significantly with PR level. In addition, we showed that tissue concentrations of VEGF and its receptors of both types in the primary tumor were in a significant positive correlation between each other, which indirectly indicated an auto/paracrine role of VEGF signal pathway in breast cancer. This observation was confirmed in subsequent dynamic study in 30 patients with locally disseminated breast cancer, examined before and after neoadjuvant chemoradiotherapy [14,15]. We also showed in this study that the degree and direction of changes in VEGF and VEGFR-2 in the course of preoperative therapy depended on the initial levels of these proteins, while their level after therapy depended on the intensity of therapeutic pathomorphosis.

In addition to breast cancer, we studied VEGF expression in tumors of other location: endometrial, esophageal, gastric, and renal cancer [3,13,20]. A common feature of all these tumors was a positive relationship between tissue VEGF levels and tumor dissemination and their direct correlations with the content of uPA and PAI-1. This indirectly confirmed the involvement of these components of plasminogen activation system in neoangiogenesis.

Attempts at using serum or plasma VEGF levels as adequate replacement of tissue expression of this protein for evaluation of angiogenesis intensity and prediction of breast cancer outcome and/or treatment efficiency have in fact failed. In our studies the correlation between tissue and serum VEGF levels was either zero or weak, blood VEGF level after removal of the primary tumor reducing in just half of the patients [12]. No correlation between tissue and circulating concentrations of VEGF and VEGFR-2 were detected in renal cancer [20].

Modern high-technology approaches to studies of the molecular marker complex. Using the microchip technology, it is possible to simultaneously evaluate the amplification, expression, or transcription of many thousands of genes and create the so-called

"molecular portraits" or "gene signatures" of tumors. The appearance of these high-technological methods, characterized by tremendous velocity and high sensitivity, due to which it is possible to work with very small volumes of material, is expected in the nearest future to produce a revolution in studies of biological markers. The drawbacks of these methods is primarily the impossibility to standardize evaluation and interpretation of the results of simultaneous evaluation of a great number of parameters.

The best studied in this sphere, similarly as in studies and clinical use of virtually all individual biological markers, is breast cancer. During just recent two years more than 70 papers were published on the possibility of clinical use of "gene expression profiles"or "molecular portraits" of breast cancer. By the present time several systems of this disease classification, based on the "gene signatures" (limited set of genes, using which it is possible to detect groups of patients with different prognosis and sensitivity to different therapies) were developed [29,37,43,44,48,50,53]. Estimation of the clinical significance of authors' own and other results varies from unconditional optimism (even prediction of the "end of surgical era" in the treatment of breast cancer) [49] to skeptical analysis emphasizing the great variety of the suggested "signatures" and impossibility to reproduce them and the numerous methodological difficulties, arising in realization of these fine methods and interpretation of their results [47]. Practically speaking, none of the "gene signatures" was included into internationally acknowledged recommendations yet [49]. One of the most famous systems based on analysis of expression of 70 genes is the Amsterdam 70-gene signature (MammaPrint™). Clinical trials of this system are now carried out within the framework of the International Program "Microarray for Node Negative Disease May Avoid ChemoTherapy" (MINDACT) [52]. The efficiency of classification based on this "gene signature" will be compared in this prospective randomized study with the efficiency of clinical criteria, based on the Adjuvant Online program. It is assumed that in order to obtained reliable results, the program should include at least 6000 patients. One more sufficiently standardized system is the signature consisting of 21 genes (Oncotype DX). Though it is already used in several foreign clinics for the formation of prognostic indexes and correction of therapeutic protocols, it is not yet included in the number of obligatory tests recommended by international organization. Wide-scale clinical trials of this system are now carried out within the framework of the USA Program Trial Assigning IndividuaLized Options for Treatment/Rx/ (TAILORx). The study is carried out in more than 10,000 women treated at 900 medical institutions of the USA and Canada [43,52].

Hence, due to progress in biochemistry, molecular biology, and biotechnology, the armory of scientists and clinicists is supplemented by a great number of biologically significant characteristics, which can be helpful in prediction and choice of adequate therapeutic strategy and protocols for the treatment of malignant tumors. However, evaluation of the majority of molecular markers did not yet become the routine practice, which is explained by high price of these studies, difficulties in interpretation of the data of simultaneous analysis of many prognostic factors, and insufficient level of proof for the majority of tests. At present only few biological markers of breast cancer are recommended for routine clinical use by the international organizations. The efficiency and usefulness of these markers is sufficiently well proven. These are: 1) ER and PR tests in all primary patients in order to determine the prospective efficiency of endocrine therapy; 2) evaluation of HER-2/neu expression (gene amplification) in patients with disseminated cancer in cases when Herceptin therapy is planned or in patients with early cancer — for including in the complex of prognostic factors; 3) the possibility of evaluating the tumor concentration of PAI-1 and/or uPA and of two "gene signatures" for detecting groups at a high risk of relapses and metastases among patients with early stages is now undergoing the final evaluation. Evaluation of biological markers in tumors of other location is limited by protocols for clinical studies of drugs of molecular direction.

It is noteworthy that the advent of new highly productive technologies, allowing simultaneous analysis of tens or even thousands of parameters in a single (very small) specimen, makes the choice of an optimal set of informative tests a priority. These tests should provide the maximum treatment efficiency for every patient at the minimum cost. One more important task is development of adequate algorithms for interpretation of the results of this complex analysis.

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