Contribution of Molecular Biology to Cancer Diagnosis and Treatment

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1. Introduction

A broad application of molecular biology techniques and new approaches in studies on pathogenesis of cancer resulted in a better understanding of the molecular and cellular events during induced and natural oncogenesis. The role of inherited mutated genes in the development of some human cancers indicates the essential role of changes in oncogenes, growth factors and tumor suppressor genes in this process, which requires several, at least more than two, mutations to develop cancer. An active engagement of scientists representing not only biomedical disciplines has significantly enriched the spectrum of research techniques widely used in the studies on the basic aspects of cancer, extended the range of methods applied in cancer diagnosis and prognosis and indicated new targets and techniques in anticancer treatment.

However, in spite of the significant progress in our current understanding of the mechanisms of pathogenesis, especially of the early events, defined as the initiation and promotion phases of cancer, the diagnosis of this disease, which develops without any distinct clinical symptoms is very often delayed. To be diagnosable clinically detectable tumors of internal organs have to be about 1 cm of diameter, which means that the number of tumor cells is about $1x10^9$ (bilion). At this stage of the disease the process is no more local. Tumor cells are usually disseminated and their population is heterogeneic, mostly because of genetic instability. The results of either local or systemic treatment of the majority of patients with advanced cancer are far from expectations.

It is a commonly believed that in addition to the elimination of a local tumor by surgery, complementary treatment such as killing the sensitive tumor cells by irradiation or systemically applied cytostatics, should be ap-

plied. Such complementary treatment could include genetic manipulation in the expression of some properties of tumor cells and/or activation of the function of host's anticancer surveillance mechanisms.

In my short introductory lecture I will try to outline in a very selective and simplified way the current problems of cancer pathogenesis, which should be well understood and considered in the evaluation of the current application of molecular biology techniques to diagnostic and prognostic methods and especially in planning of new therapeutic approaches including immunogenotherapy as well as anticancer genotherapy and in an estimation of their potential and real effectiveness in the treatment of cancer patients.

2. Malignant transformation — initiation phase of neoplastic growth

A genetic basis for cancer development has been hypothesized for nearly a century and has been supported by familial, epidemiologic and cytogenetic studies. Only in the last decade a direct evidence has been provided through the demonstration of both inherited and somatic mutations in tumor cells. A current view is that cancer results, at least in part, from mutations in two classes of genes:

1) oncogenes, which when mutated, act in a positive fashion to facilitate and accelerate tumor cells proliferation, and

2) suppressor genes which when mutated loose their ability to suppress tumor growth.

As the oncogenes are involved in the cell growth regulatory circuits, on many different levels the tumor suppressor genes can be predicted to function in a variety of pathways of cell growth control. Activation of oncogenes by regulatory or structural changes can contribute to the intrinsic growth stimulation of the cells — tumor suppressing genes can facilitate tumor growth if they are mutated or lost. Another gene group may influence progression — various genes that can modulate growth of already established tumors e.g. by influencing their invasive or metastatic behavior, or their sensitivity/resistance to cytostatics and immune rejection.

The cellular counterparts of at least some of the retrovirally transduced oncogenes, have been incremented in the natural oncogenic process. Activated ras-oncogenes have been found in carcinomas of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, stomach, hematopoetic tumors of lymphoid or myeloid origin, fibrosarcomas, rhabdomyosarcomas, melanomas, neuroblastomas, gliomas and teratocarcinomas (Tab. 1).

TABLE 1
SELECTED MAJOR RETROVIRALLY CARRIED ONCOGENES

Oncogen	Pathogenicity	Localization, functions	Involvement of cellular counterpart in human tumors
v-abl	B-cell limphoma fibrosarcomas	Membrane, thyrosine-kinase	bcr/abl translocation in CGL, ALL
v-erb B	Erythroleukemia, fibrosarcomas	Membrane receptor for EGF	Amplified in squamous cell carcinoma and glioblastoma
v-mil/raf	Sarcomas	Cystolic serine/threonine kinase	Rearrangement in primary stomach cancer
v-myb	Myeloblastosis	DNA-binding nuclear regulatory protein	Amplification in colon cancer, AML
v-myc	Carcinomas, Leukemias, Sarcomas	DNA-binding nuclear regulatory protein	Translocation to Ig locus in Burkitt lymphoma (c-myc) Amplification in carcinomas (C-, N- or L-myc) neuroblastomas (N-myc) plasma-cell leukemia (c-myc)
v-ras	Sarcomas	Membrane inner surface assoc., signal transducing G protein	Mutational activation in many carcinomas and leukemias, occasional amplification in carcinomas

(acc. to 1

Approximately 10-25% of all tested human cancer have activated K-ras genes, although in certain tumors, such as colon carcinomas, the frequency may be as high as 40-50%.

The variety of structural and regulatory changes by mutations, translocation, retroviral insertion and amplification that can activate the same oncogene in different naturally occuring tumors shows a direct connection between the activated gene and the neoplastic phenotype, independently of the mechanism of activation.

All known protooncogene functions are involved in the control of cell division and they can be classified under the following headings: — growth factors, receptors, non-receptor kinases, transcriptional factors, nuclear proteins (1).

In the past few years, several tumor suppressor genes, called antioncogenes (TSGs), have been isolated by molecular cloning techniques. In some cases these genes are lost or inactivated in the germline and their inactivation may predispose to susceptibility to tumor formation. More often, TSGs are functionally inactivated by somatic mutation. While the normal cellular function of any of TSGs identified to date is not yet understood, it seems likely that they function in some manner to regulate the normal growth and

differentiation of cells. Some of the products of TSGs could be capable of generating negative growth regulatory signals. In other cases, gene products may function as transducers of normal growth inhibitory signals (2).

The predisposition to the development of many human cancers including rare tumor syndromes, such as retinoblastoma, neurofibromatosis and multiple endocrine neoplasia and common human cancers, such as colon, breast and ovarian tumors, may be inherited (Tab. 2).

TABLE 2
EVIDENCE FOR TUMOR SUPPRESSOR GENE ALTERATIONS IN SELECTED HUMAN TUMOR TYPES FROM LINKAGE ANALYSIS (LA), LOSS OF HETEROZYGOSITY ANALYSIS (LOH) OR THE DETECTION OF SPECIFIC MUTATIONS

Tumor type	Chromosome region	Evidence
Retinoblastoma	13q14	LA, LOH, RB1 mutation
Osteosarcoma	13q14, 17p13	LOH, RB1, p53 mutation
Wilms'	11p13, 11p15, others	WT-1 mutation, LOH, LA
Rhabdomyosarcoma	11p15, 17p13	LA, LOH
Bladder (TCC)	9, 11p, 17p13	LOH, p53 mutation
Hepatoblastoma	11p	LOH
Lung (SCLC)	3p, 13q14, other	LA, LOH, RB1, p53 mutation
Colorectal	5q, 17p13, 18q, others	LA, LOH, p53, DCC mutation
Breast	11p, 13q14, 17p13, others	LOH, RB1, p53 mutation
Glioblastoma	10q, 17p13	LOH, p53 mutation
Neuroblastoma	1p	LOH
Neurofibromatosis		
type 1	17p13, 17q	p53 mutation, LA, NF1 mutation
type 2	22q	LA, LOH
Multiple endocrine		
neoplasia type 1	11q	LA, LOH
type 2	10, 1	LA, LOH

(acc. to 2)

The similarities between p53 and pRB are further extended by the recent finding that mutant p53 alleles can be passed through the germline where they may serve as congenital determinants of cancer predisposition.

According to the Knudson's concept, both alleles of the cancer predisposing gene must be inactivated for the cell to gain a proliferative growth advantage. In the hereditary form of cancer, one allele is inherited in a

mutant form in all cells. If a somatic mutation of the allele has been inherited from the nonaffected parent, tumor initiation and progression ensues (2).

Continued identification of the TSGs involved in these predisposition syndromes should lead to more effective methods for the identification and clinical management of individuals and members of the family who are at an increased risk of developing tumors, especially of breast, ovary and colon.

Further identification of the alterations in both oncogenes and TSGs that indicate tumor development, resulting in, a description of the molecular basis of cancer, should provide clues to develop new methods:

— to prevent the disease, by genetic manipulation or chemoprevention programs,

— to detect the disease at its early — more easily curable stages,

— to develop new chemo- (immuno-, geno-) therapeutic agents that might selectively inactivate mutated oncogene products or that might mimic or restore the normal biological action of suppressor genes (genotherapy).

By inserting wild type suppressor genes into the tumor cells that lack them, one may be able to reinstate a semblance of normal growth control, and with this, cause the reversion of tumor cells to the normal growth pattern. Early success had been reported, including the use of retrovirus transducing vectors to insert wild-type RB gene copies into osteosarcoma and prostate carcinoma cells that lack them. These genetically reconstituted cells grow more slowly *in vitro* and loose tumorigenicity in nude mice. Insertion of an entire chromosome 11 resulted in a loss of tumorigencity in Wilms' tumor cell, as demonstrated many years ago, before modern techniques of gene transduction into tumor cells were developed (2).

3. Transition from local neoplastic alteration to disseminated phase of tumor disease

The following stage in cancer development is hyperplasia, i. e. an enlargement of the size of tumor population as a consequence of an increased number of proliferating cells or rather clone(-s) of transformed cells with the inherited alterations in their genetical material. These alterations determine their immortality, and, in addition, their limited sensitivity or resistance to growth controlling signals. The critical point is the size of primary tumor — as long as the tumor mass does not exceed 2-3 cm, the process of diffusion seems to be sufficient for the exchange of alimentary and metabolic products as well as uptake of the regulatory factors present in the tissue fluids. With the increasing size of the tumor, its central part may be hypooxidated, and some cells will undergo necrosis.

The next important step is the release of proteolytic enzymes which results in the alteration of spatial structure of the tissue, where the tumor is located. Increased proteases levels in the tumor tissue occur either in tumor cells, normal cells intermixed or adjacent to the invasion site by their over

expression. The disturbed balance between the active enzyme and tissue inhibitors of metallo-proteases determines if the local matrix degradation will occur. Although enhanced by tumor cells, proteolysis is still regulated in a temporal and spacial fashion with respect to cell attachment and migration. Proteolytic activity depends on the balance between local concentration of activated enzymes and their endogeneous inhibitors (3).

There is a functional similarity between tumor invasion and angiogenesis. Transition from *in situ* to invasive carcinoma is associated with the dissolution of the basement membrane and migration into the interstitial stroma, which contains stromal cells and matrix molecules, including adhesion molecules, growth factors, cytokines, angiogenic factors, etc.

Early phases of angiogenesis, stimulated by tumor cells excreating angiogenic factor necessary to supply tumor cells through the newly formed capillary vessels, involve enzymatic dissolution of the parent vessel basement membrane and endothelial migration into the stroma forming the sprout toward angiogenic stimulus. Lateral proteolysis of the stroma permits expansion of the sprout diameter and lumen formation. Angiogenic factors, such as basic Fibroblastic Growth Factor (FGF), induce endothelial cell migration, proteolysis and proliferation. Several endogeneous and exogeneous angiogenic and antiangiogenic factors produced by tumor and normal cells have been defined and the old Folkman's hypothesis (4) that the invasive tumor growth is directly related to the degree of angiogenesis has been now well documented (Tab. 3).

TABLE 3
ENDO- AND EXOGENEOUS SUBSTANCES WITH EFFECTS ON VASCULAR PROLIFERATION

Angiogenic Activity	Anti-Angiogenic-Activity
aFGF	TGFβ
bFGF	α Interferon
ESAF	TNFγ
TNF	TIMP 1
TAF	TIMP 2
Prostaglandins	Protamine
PGE1, PGE2	Cartilage extract
Angiotropin	Hydrocortisone
Angiogenin	Heparin
Angiotensin	Retinoids
Heparin	Methotrexate
Histamine	Mitoxantrone
Lactic acid	Pencillamine
Leukotriene B4	Fumagillol Angiostatin

A better understanding of the role of proteolysis and angiogenesis in cancer progression and metastasis also resulted in the application of several new methods in the improved diagnostic and prognostic approaches as well as indicated new targets for therapeutic strategies directed not only to eliminate or destroy tumor cells, but also to influence the host factors contributing to the tumor growth. The tables presented below show some examples of measuring the neoangiogenesis as a prognostic clinical marker (Tab. 4) and enzyme estimation in some tumors as prognostic factors (Tab. 5).

TABLE 4
NEOANGIOGENESIS AS A PROGNOSTIC CLINICAL MARKER

Breast cancer[1,2]
Malignant melanoma[3]
NSCLC[1]
Prostatic cancer[3]
Gastric cancer[3]
Rectal cancer[3]
Oral cavity cancer[3]
Cervical cancer[3]
Urinary bladder cancer

[1]CD31 Ab
[2]VEGF-mRNA
[3]F-VIII RAg

(acc. to 5 – 14)

Spacial alterations in tumor and surrounding tissue microenvironment, bringing closer the newly formed blood vessels unable the tumor cells to move and leave the primary tumor site. At this stage the neoplastic process is no more local. The disseminated growth begins when the interactions between tumor cells and normal cells present in the direct surrounding takes place. These interactions, called intercellular dialog or intercellular communication between tumor cells, fibroblasts, endothelial cells and cellular components of immune system, are being engaged. In addition to direct intercellular interactions several soluble factors produced or released from interacting cells or present and released from the extracellular matrix are being involved (15,16).

TABLE 5
PROTEOLYTIC ENZYMES AS PROGNOSTIC MARKERS*

Type of Tumor	Enzyme
colorectal cancer	Interstitial collagenase
lung cancer	Interstitial collagenase Stromelysin 3, Gelatinase B
head and neck cancer	Stromelysin 1, 2 and 3
gastrointestinal cancer	Matrilysin (transcipts)
prostatic cancer	Matrilysin (transcipts)
breast, colon, gastric, prostate cancers	Gelatinase A
breast cancer	Stromelysin 3, Gelatinases

*Methods of detection in tumor tissues or in serum:
— Immunoperoxidase staining for localisation of MMPs,
— Northern blot analysis of MMP transcript in RNA samples, *in situ* hibridisation (ISH) of MMP transcripts,
— MMP levels in the body fluids.

(acc. to 15)

These include adhesion molecules, growth factors such as cytokines and, hormones. The interactions of these factors with specific membrane receptor trigger a cascade of intercellular biochemical signals, resulting in the activation, or repression of various subsets of cellular genes, including those involved in mitogenic growth signaling. The examples of cytokines with implicated angiogenic activity are listed in Table 6 which also shows selected types of human tumors in which the detection of cytokines has been used as a prognostic marker (16).

TABLE 6
CYTOKINES WITH IMPLIED ANGIOGENIC ACTIVITY

Expressed in *in vitro* Propagated Human Tumor Tissues and/or Cells:		
VEGF	bFGF, EFG	TGFα
TNFα	TNFβ	PLEOTROPIN
MIDKINE	PDECGF	HGF/SF

(acc. to 16)

Cytokines as Prognostic Marker	
Type of tumor	Cytokin
Ovarian cancer	CSF-1, uPA
Gynecological tumors	IL-6
Breast cancer	TGFα, TNFα
Leukemias	s IL-2R, s CD8 and ICAM-1

(acc. to 17 – 20)

A number of specific cell surface associated molecules that modulate cell to cell and cell to extracellular matrix adhesion interactions have been characterized. These include: integrins, cadherins, laminins, IgG superfamily, CD44, etc. The role of this class of molecules in tumor growth, invasiveness and metastases is under intensive investigation. Tumor cells must show both decreased cell and matrix adhesive properties (e.g. to detach, leave and move from the primary site) as well as an enhancement of these functions at various stages of the multistep metastatic process. During various steps of this process, tumor cells must get into the blood stream, attach to vascular endothelium, leave the vessel and settle in a new localisation, where the secondary, metastatic growth may be initiated (21,23).

Among the mentioned examples of adhesion molecules involved in tumor invasive growth and metastasis, CD44 deserves special interest. It is a transmembrane glycoprotein with 90 kDa molecular weight present in normal cells. It is known as "lymphocyte homing receptor", "extracellular matrix receptor" or "hyaluronate receptor". Using various molecular techniques different posttranscriptional isoforms of this molecule were defined. The CD44 variant 5 and 6 are of special interest. In activated lymphocytes the standard form of this molecule is substituted by isoform CD44 variant 6, which enables the activated cells migration from peripheral blood vessels to corresponding lymphatic vessels and their settlement in the regional lymph node (24). Similarly, the metastatic cancer cells, spreading along the lymphatic vessels, are expressing the same variant form of this molecule, thus imitating the normal activated immune cells, temporarily expressing these adhesion molecules when they are leaving blood vessels and migrating to lymph node during the inflammation. As it is shown in Table 7, several authors are using the determination of CD44 isovariant forms as a prognostic marker in several types of human tumors.

The following level of tumor-host interactions during the disseminated phase of cancer disease is the confrontation of tumor cells with the cellular and humoral components of the immune system. One of the central concepts of tumor immunology is that cancer cells posses antigens that are not found on normal tissues and whose presence allows the host to recognize a tumor as a foreign tissue. Evidence for the existence of tumor associated antigens that mediate the immunologic rejection of tumor cells has been obtained from the animal model experiments. Several categories of tumor-associated antigens (TAA) have been demonstrated in human tumors, some of them are known to be major histocompatibility complexes of (MHC) normal or modified antigens, majority of them are expressed not only in tumor cells but also on normal cells. However, the differences are quantitative or the antigens on normal cells are expressed only during certain, usually early phases of development (e.g.carcino-embryonal — CEA — or oncofetal antigens). More recently, several immunogenic oncogenic proteins have been defined using the molecular biology techniques. The overexpression or amplification of genes coding for these oncogenic proteins has been detected in various animal and human tumors (29).

TABLE 7
ADHESION MOLECULES INVOLVED IN TUMOR INVASION AND METASTASIS

1. Integrins
2. Laminin, 67-69 kDa laminin-elastin binding protein
3. Cadherins
4. Immunoglobulin superfamily IgSF
5. CD44

Adhesion Molecules as Prognostic Marker

CD44 Isovariant Forms:
Breast cancer*
Urinary bladder cancer*
Colorectal cancer*,** (30.8 ± 11 nM)
Gastric cancer** (24.2 ± 9.8 nM)
Brain metastases of cancer*
NSCLC*
Large cell lymphoma*

*increased level in tumor cells; **increased level in patients' serum.

(acc. to 25 — 28)

TABLE 8
IMMUNOGENIC ONCOGENIC PROTEINS

Name	Tumors in which overexpression or gene amplification occurs
HER-2/neu[1]	Human carcinoma of breast, uterus, ovary, stomach, lung (adenocarcinoma), 20 – 40% ductal breast carcinomas, 60 – 80% ductal carcinomas *in situ*
p21 ras[2]	Mouse and human tumors (20%), incl. 90% pancreatic adenocarcinomas, 60% follicular carcinomas of the thyroid, 50% colon adenocarcinomas, 40% myeloid leukemias
p210-bcr-abl[3]	95% human chronic myelogenous leukemia (CML), 10% children and 25% adults with accute lymphocytic leukemia (ALL)

[1]member of EGFs family, functions as EGF receptor, overexpressed as a result of gene amplification; [2]prototype of oncogenic protein, that is a result of somatic DNA mutation (single aminoacid substitution and abberant protein function); [3]translocation of c-abl protooncogen from chromosome 9 to the specific breakpoint cluster (brc) region on chromosome 22. Formation of a bcr-abl fusion gene, that encodes at 210 kD chimeric protein with abnormal thyrosine kinase activity.

(acc. to 29)

Another example of demonstration of the expression of MAGE-1,3- and BAGE genes tested by PCR amplification of reverse transcribed RNA extracted from various human tumor samples has recently been published by van Pel et al. (30). Using the antigens obtained by genetic engineering methods for immunisation *in vitro*, several clones of T lymphocytes were obtained which recognize these antigens on various human melanoma cells and other tumor cells (Tab. 9).

TABLE 9
EXPRESSION OF THE *MAGE-1,-3* AND *BAGE* GENES

Histological type	Number of tumor tested	Percentage of tumors positive cells		
		MAGE-1	MAGE-3	BAGE
Melanomas	178	36	65	22
primary lesions	38	14	36	8
metastatic lesions	140	42	74	26
Bladder carcinomas	62	19	34	15
superficial tumors	32	7	14	0
infiltrating tumors	30	36	57	30
Mammary carcinomas	79	18	11	10
Head and Neck squamous cell carcinomas	53	25	48	8
Non small cell lung carcinomas	64	34	31	6
Sarcomas	18	11	22	6
Prostatic carcinomas	22	15	15	0
Colorectal carcinomas	42	0	16	0
Renal carcinomas	50	0	0	0
Leukemias and lymphomas	0	0	0	0

Expression was tested by PCR amplification of reverse transcribed RNA extracted from tumor samples.

(acc. to 30)

In spite of the presence of TAAs and quite well defined immune humoral and cellular effector antitumor responses, in the majority of patients the cancer disease develops progressively, and is mortal because of the metastases.

4. Anticancer immuno- and immunogenotherapeutic approaches and strategies

From the begining of this century several approaches to immunotherapy of cancer have been used. Attemps were made to stimulate endogeneous anti-tumor immunity within the host through the administration of bacterial products, chemically defined immunomodulators, cytokines and vaccines. In passive immunotherapy antibodies or lymphoid cells (as adaptive immunotherapy), have been applied, providing exogeneous immunity. The attemps to apply immunotherapy in cancer patients are currently based mostly on empirial observations.

Further development of these approaches requires a better understanding of the components involved in the regulations of the immune system. During the last two decades somatic cell hybridisation has facilitated the production of large amounts of monoclonal antibodies recognizing a great variety of epitopes of TAAs and recombinant DNA technology has provided novel recombinant cytokines and vaccines (31,33).

The initial development of cytokines application in cancer immunotherapy involved systemic administration of pharmacologic doses of a given recombinant protein. By early 1980, IL-2 had been recognized as a major T cell growth activation factor. Furthermore, it appears to be a potent growth and activation factor of NK cells, which were originally thought to represent a distinct population of lymphocytes, specifically endosed with the ability to recognize and to kill tumor cells (32).

Tremendous efforts in the evaluation of systemic IL-2 administration for anticancer therapy ensued and this approach still continues. In general, clinical results with systemic IL-2 administration have been modest and the systemic toxicity is often severe. Nonetheless, there is a documented response rate to systemic IL-2 of roughly 20% patients with metastatic kidney cancer and melanoma (34).

IFNα has been most thoroughly examined with regard to efficacy and toxicity. Particularly impressive results have been observed in hair cell leukemia, chronic myelogenous leukemia and nodular non-Hodgkin's lymphoma. Among the solid neoplasms that respond to IFNα are Kaposi's sarcoma, glioblastoma, melanoma and kidney cancer. Interlesional or regional administration of IFNα has produced objective tumor regression in basal cell carcinoma, bladder and ovarian cancers (35).

Application of the recombinant DNA technology has allowed for the isolation of genes that encode certain tumor associated peptides. Several of these genes have been incorporated into vaccinia virus and other potentially immunogenic viruses.

Genes for different cytokines (Tab. 10) including IL-2, IL-4, IL-6, IL-7, IL-12 and IFNγ have also been introduced into tumor cells, potentiating specific antitumor immunity in animal models. The efficacy of tumor cell vaccines is likely to depend upon further definition of human tumor specific

transplantation (resistant) antigens as well as a better understanding of the peptides that are actually recognized by antigen presenting cells inducing the effector functions of T cells (31).

TABLE 10
PARACRINE CYTOKINE ADJUVANTS IN CANCER IMMUNOTHERAPY

Cytokin	*In Vivo* Effects of Cytokine Gene-Transduced Tumors
IL-2	— regression of transduced tumors, local infiltration of cytotoxic lymphocytic cells (CD8+), — transplantation immunity (challenge with parental tumor), — correlation between ↑ level of local IL-2 production and local and systemic antitumor response
IL-4	— regression of transduced tumors, massive infiltration of MØ and eosinophils, — transplantation immunity, systemic antitumor response dependent on CD8+ and partially CD4+ cells, enhanced presentation of tumor antigens by MØ to CD4+ cells and ultimate stimulation of CD8+ cytotoxic lymphocytes
IL-6	— pleomorphic local inflammatory infiltrate (monocytic and lymphocytic cells), — moderate vaccine effect even with poorly immunogenic tumors
IL-7	— systemic antitumor immune responses MHC class II + (transduced) plasmocytoma line, — immunity dependent on CD4+ and CR3+ cells (MØ), not on CD8+ cells
IL-3	— local inflammatory infiltrate, systemic antitumor immunity dependent on both CD4+ and CD8+ cells
IL-12	— proinflammatory cytokine. induced production of IFNγ, activated cells producing GM-CSF and TNFα, also NK, CD8+, CD4+ cells, — transduced tumor cells — antitumor, antimetastasis effect
IFNγ	— induces or upregulates both MHC class I and MHC class II gene products, rejection of transduced tumors, systemic transplantation immunity
TNF	— transduced tumor cells grow more slowly *in vitro*, sometimes are rejected (direct effect on tumor cells or through activation of MØ), Immunisation does not generate systemic immunity
GM-CSF	— transduced cells induce long-lasting systemic antitumor immunity, dependent on both CD4+ and CD8+ cells (despite the fact that the tumor cells were MHC class II) – [promotion of differentiation of hematopoetic precursors to dendritic APC cells (?)]
G-CSF	— local inflammatory infiltrate, large number of MØ, no systemic immunity documented
MCP-1	— local inflammatory infiltrate, large number of MØ, no systemic immunity documented

Under physiologic conditions, appropriate cytokines are produced in high amounts locally, near to the site of antigen source (tumor cells!), often acting in concert with antigen derived signals to generate immune response.

In the last 5 years, the fields of cancer vaccines and cytokines have been combined in new approaches that seek to express specific cytokines local to the site of tumor. It was first suggested to exploit the paracrine physiology of cytokin action with the hope of generating more potent and antigen-specific immune responses against cancer (31). Table 11 ilustrates various strategies of immunotherapeutic anticancer treatment. It also shows the location — among other approaches — of cytokines used either as recombinant preparations (point 3) or as vaccines, in which the tumor or normal cells were transfected with the gene for a given cytokine (point 4 c).

TABLE 11
STRATEGIES OF IMMUNOTHERAPEUTIC ANTICANCER TREATMENT

Active Immunotherapy
1) bacterial immunomodulators
2) chemically defined immunomodulators
3) cytokines — systemic or local injections of recombinant preparations
4) vaccines — autologous or allogeneic tumor cells modified *ex vivo* to augment their immunogenicity:
a) heterogenisation (forerunner to paracrine cytokine adjuvants)
— virus infected tumor cells or their lysates
— transduction of tumor cells with specific viral gene
b) introduction of allogeneic MHC genes into tumor cells
c) transfection with genes for various cytokines
Passive Immunotherapy
1) anti TAAs antibodies, incl. monoclonal (mAbs)
2) antiidiotypic antibodies
3) cytostatic-, toxin-, radionuclide-conjugated mAbs (specific carriers)
4) adaptive therapy — isolated lymphocytes or TIL, *ex vivo* activated with e. g. IL-2 (LAK cells) alone or combined with administration of cytokine

I presented how much we do learn and understand about the pathogenesis of cancer, especially of its initiation and promotion phase of development, how much the current molecular biology techniques enriched the diagnostic and prognostic possibilities and finaly how much we have achieved owing to the experimental tumor models. But believe me, I could easily list the unanswered questions and serious problems the oncologist is facing at the bedside of any cancer patient, especially while considering predicting the natural course of the disease and designing therapeutic pro-

gram. I would like to end with the opinion of Danie Calleman, who said: *The greater the power of science to intervene in nature animate and inanimate, the greater possibility of doing harm as well as good.*

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Contribution of Molecular Biology to Cancer Diagnosis and Treatment

Summary

A broad application of molecular biology techniques and new multidisciplinary approaches in studies on cancer pathogenesis resulted in a better understanding of the early events in natural and induced oncogenesis. In spite of significant improvement in diagnostic and prognostic techniques and possibilities, as well as in the development of new therapeutic strategies, the effects of cancer patients treatment are far from expectations. The aim of this lecture is to find a bridge between a current understanding of early events during oncogenesis and the complexity of cancer disease, whose development and course is a consequence of interactions on various levels, local as well as systemic between cancer cells and cellular components of patients surveillance and homeostasis systems.

Key words:
cancer, diagnosis, therapeutic strategies.

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