

Natural mechanisms protecting against cancer

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Abstract

Carcinogenesis is a multistage process. At each step of this process, there are natural mechanisms protecting against development of cancer. The majority of cancers in humans is induced by carcinogenic factors present in our environment including our food. However, some natural substances present in our diet or synthesized in our cells are able to block, trap or decompose reactive oxygen species (ROS) participating in carcinogenesis. Carcinogens can also be removed from our cells. If DNA damage occurs, it is repaired in most of the cases. Unrepaired DNA alterations can be fixed as mutations in proliferating cells only and mutations of very few strategic genes can induce tumor formation, the most relevant are those activating proto-oncogenes and inactivating tumor suppressor genes. A series of mutations and/or epigenetic changes is required to drive transformation of a normal cell into malignant tumor. The apparently unrestricted growth has to be accompanied by a mechanism preserving telomeres which otherwise shorten with succeeding cell divisions leading to growth arrest. Tumor can not develop beyond the size of 1–2 mm in diameter without the induction of angiogenesis which is regulated by natural inhibitors. To invade the surrounding tissues epithelial tumor cells have to lose some adhesion molecules keeping them attached to each other and to produce enzymes able to dissolve the elements of the basement membrane. On the other hand, acquisition of other adhesion molecules enables interaction of circulating tumor cells with endothelial cells facilitating extravasation and metastasis. One of the last barriers protecting against cancer is the activity of the immune system. Both innate and adaptive immunity participates in anti-tumor effects including the activity of natural killer (NK) cells, natural killer T cells, macrophages, neutrophils and eosinophils, complement, various cytokines, specific antibodies, and specific T cytotoxic cells. Upon activation neutrophils and macrophages are able to kill tumor cells but they can also release ROS, angiogenic and immunosuppressive substances. Many cytokines belonging to different families display anti-tumor activity but their role in natural anti-tumor defense remains largely to be established. © 2003 Elsevier B.V. All rights reserved.

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1. Introduction

Natural defense mechanisms protecting against infection or against cancer can be categorized into non-immune and immune with the latter belonging either to innate or adaptive immunity.

Our concepts of the role of the immune system in the protection against cancer usually belong to two extreme attitudes that either exaggerate or negate the importance of immunity in this defense. On the one hand, Burnet suggested that immunosurveillance is responsible for detecting and eliminating tumor cells being a central mechanism by which tumor development is kept in check [1]. Based on this hypothesis, it could be assumed that arising tumor cells are

destroyed by the immune system in the majority of cases, probably hundreds or thousands times during our life, and only rarely they evade this defense giving rise to malignant tumors. On the other hand, immune stimulation hypothesis predicts that low immune reactivity against tumors, as it is usually observed in cancer patients, is stimulating rather than inhibiting tumor cells [2]. As it often happens with extreme attitudes neither of the above theories seems to be correct. Immune defense mechanisms form the last barrier in our natural mechanisms of protection against cancer and are probably less effective as compared with some other mechanisms operating at earlier stages of malignant tumor formation.

The immune mechanisms that protect against infectious microorganisms, operating during the entire lifespan of individuals including their reproductive periods, have undergone positive selection and thus became perfected during human evolution under selective pressure of attacking microbes and diseases they produce. Non-immune mechanisms protecting

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against cancer, as they are active at early stages of the multistep process of carcinogenesis, had similar chance for positive selection and perfection. It should be kept in mind that genetic selection acts on the individual phenotypes and either favors or hinders reproduction and thus the propagation of that individual's genotype [3]. Thus, selection may operate at any time from conception to the end of the reproductive period but not beyond that time [4]. Since the multistage process of carcinogenesis and full development of cancer lasts for up to several decades [5] and cancer incidence grows exponentially with age, it can be hypothesized that immune anti-tumor mechanisms operating usually in elderly population did not have such an opportunity for positive selection and improvement in our evolution. Some observations seem to support indirectly this hypothesis suggesting more important role of non-immune anti-carcinogenic mechanisms operating early as compared with anti-tumor immunity operating later. Nucleotide-excision repair (NER), which is only one of the DNA repair mechanisms, protects against cancer at early stage of carcinogenesis. Impairment of NER observed in the human recessive hereditary disease *xeroderma pigmentosum*, causes a 5000-fold increase in the incidence of squamous and basal cell carcinoma and a 2000-fold increase in the incidence of melanoma [6]. On the other hand, prolonged treatment with immunosuppressive drugs in allograft recipients effectively impairs adaptive immunity but increases the risk of squamous and basal cell carcinoma "only" 65-fold according to one of the reports [7] although skin cancers are among the most frequent neoplasms in allograft recipients [8]. In another report, incidence of cancer of the lip, skin (non-melanotic), kidney, endocrine glands, and cervix of uterus, was increased 10–30 times in renal transplant patients [9].

Our knowledge on the natural defense against cancer is far from being complete and is full of paradoxes and misconceptions. A good example of this unawareness was our conviction that supplementation of human diet with β carotene should diminish the incidence of cancer (see below).

2. The multistage process of carcinogenesis

Based on the experimental studies in rodents, the multistage process of carcinogenesis has been traditionally divided into initiation, promotion and progression [10,11] (Fig. 1).

Initiation begins with DNA damage in a cell population exposed to chemical, physical or microbial (mostly viral) carcinogens. If not repaired, DNA damage could produce genetic mutations. The majority of these DNA alterations is irrelevant to the life of the cell and is completely innocent from the point of view of cancer risk. On the other hand, the damage of critical genes could be lethal and obviously again no risk of tumor induction ensues in this case. Only occasionally, the mutations create growth advantage for a cell because of increased proliferation or reduced cell death.

The most relevant in this regard are mutations activating proto-oncogenes and inactivating tumor suppressor genes.

Promotion could be defined as a process of clonal expansion of an initiated cell that results in the formation of pre-neoplastic cells producing nodules, polyps or papillomas, and progression stage is characterized by the transformation of pre-neoplastic cells into malignant tumor invading surrounding tissues and forming metastases.

During the multistage process of carcinogenesis, only rarely selected cells could proceed through a series of genetic and epigenetic changes from one stage to the next one along the pathway leading them ultimately to the formation of malignant tumor. At each stage, the pathway leading to malignancy is only one of the possible routes and at each stage there are natural anticancer defense mechanisms further diminishing cancer risk. The peculiarity of these defense mechanisms stems from the fact that actually the most effective of them seem to operate at the very early stage preceding the formation of the neoplastic cell. The examples given below could illustrate the issue:

- Many chemical carcinogens as well as ionizing and ultraviolet radiation are carcinogenic mainly through the induction of reactive oxygen species (ROS) able to produce DNA damage. However, various natural substances, many of which are termed antioxidants, present in our food or synthesized in our organism inhibit the formation of, trap or decompose ROS preventing their carcinogenic activity.
- Toxic compounds, including carcinogens, entering our cells are detoxified by enzymes and/or are removed from target epithelial tissues by a group of membrane proteins.
- If the above mentioned mechanisms do not prevent carcinogens from gaining access to genes, damage of DNA could occur.
- Damage of DNA is probably repaired in most of the cases.
- Even if unrepaired, DNA damage can produce mutations and neoplastic transformation of only a small number of cells, usually stem cells, because the remaining cells are differentiated, do not proliferate and are committed to death.
- Only mutations of very few critical genes can induce tumor formation, the most relevant are those activating proto-oncogenes and inactivating tumor suppressor genes.
- Not a single mutation but a series of mutations and epigenetic changes is required to drive transformation of a normal cell into a malignant one and into the tumor. It is estimated that four to six genetic changes are required for the formation of a malignant tumor in humans [12].
- Even if a given cell achieved an apparently unrestrained growth due to activation of appropriate proto-oncogenes and inactivation of some tumor suppressor genes, telomeres shorten progressively with succeeding cell divisions leading to growth arrest unless telomerase is activated.
- Tumor can not develop beyond the size of 1–2 mm in diameter without the induction of angiogenesis.

MULTISTAGE PROCESS OF CARCINOGENESIS

- INITIATION**
- damage of DNA
 - mutation induction in critical target genes
 - activation of proto-oncogenes
 - inactivation of tumor suppressor genes
 - cell replication and fixation of mutation
- PROMOTION**
- clonal expansion of initiated stem cells
 - development of benign tumor
- PROGRESSION**
- altered expression of enzymes
 - proteolysis
 - adhesion & deadhesion
 - invasion
 - migration
 - metastasis

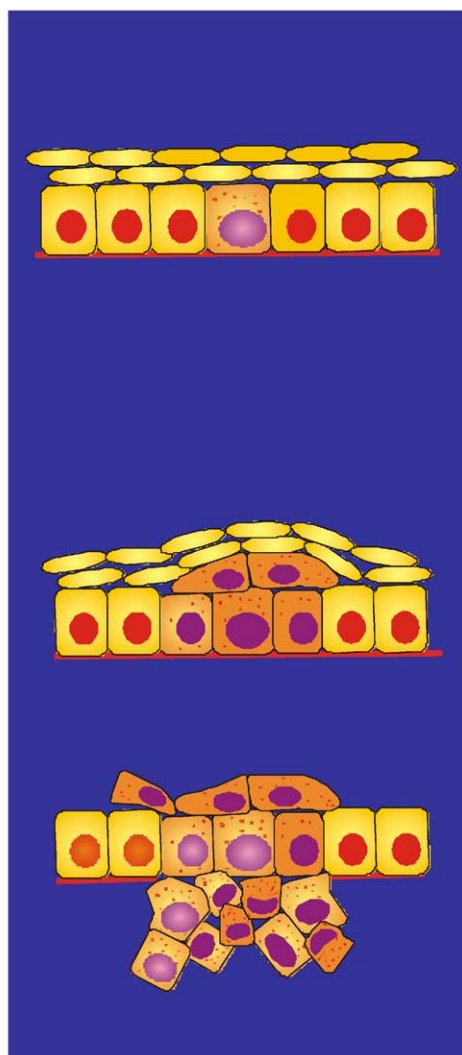


Fig. 1. Multistage process of carcinogenesis.

- Invasion and dissemination of tumor cells is prevented by some natural mechanisms. As for epithelial cells, adhesion molecules keep them bound to each other and they usually lack the enzymes able to dissolve the basement membranes.
- Tumor cells invading the surrounding tissues and penetrating lymphatic and blood vessels to disseminate and to form metastases can be recognized and eliminated by the immune system.

3. Non-immune anticancer defense mechanisms

3.1. Anti-carcinogenic substances present in the human diet

It is considered that 80–90% of all human cancers are environmentally induced [13] and 30–40% of them by carcinogens present in our diet [14]. Almost all plants in-

cluding those forming human diet, seem to produce some kind of toxic substances called natural pesticides, defending them against fungi, insects and/or animals feeding on them [15–17]. Many of both synthetic and natural pesticides are carcinogenic at appropriate doses [18]. Although our fears are mainly concentrated on synthetic pesticides we have to realize that probably every vegetable and fruit we consume contains some natural pesticides that are carcinogenic in rodent tests [15] and it is estimated that natural pesticides constitute about 99.99% of all pesticides present in human diet [15,16,18]. Most carcinogens are genotoxic. They damage DNA either directly by forming covalent adducts with DNA or indirectly after being activated from inactive pro-carcinogens or through reactive oxygen species they induce. However, some carcinogens are not genotoxic but stimulate cell proliferation thus increasing the probability of spontaneous occurrence of errors during DNA replication. Finally, some chemical bonds, including those present in DNA, can

Table 1

Some molecules present in the human diet showing anti-carcinogenic effects [17,21–26]

Anti-carcinogenic substance	Activity
Vitamin C	Exerts antioxidative effects and blocks the formation of carcinogenic nitrosamines
Vitamin A	Exerts antioxidative effects and participates in the differentiation of epithelial cells
Vitamin E	Exerts antioxidative effects
Folic acid, Vitamins B6 and B12	Participate in nucleotide metabolism and their depletion increases the risk of DNA damage and cancer
Coumarins	Induce the activity of glutathione S-transferase
Phenols	Influence the activity of carcinogen-metabolizing enzymes
Flavones	Stimulate the activity of microsomal mixed-function oxidase
Phytosterols	Affect signal transduction pathways regulating tumor growth and apoptosis
Selenium	Is present in the active site of glutathione peroxidase
Uric acid	Exerts antioxidative effects
Glutathione	Exerts antioxidative effects and participates in the inactivation of carcinogens

disintegrate spontaneously under physiological conditions. As mentioned above, one of the peculiarities of the natural anticancer defense mechanisms is that they start operating at the stage preceding the formation of tumor cells. UV and ionizing radiation as well as many chemical compounds induce formation of ROS, which could damage DNA. Among them are: superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\bullet$), singlet oxygen ($^1O_2$), alkoxy radical ($RO^\bullet$), and peroxy radical ($ROO^\bullet$) [19], with hydroxyl radical being the most active in damaging DNA. Oxidative damage may include: single- and double-strand breaks, abasic sites, and a variety of alterations in every constituent of DNA. For example, oxidative damage to cytosine alone results in at least 40 modified derivatives [20].

ROS are also formed as a result of normal cellular respiration in mitochondria, by neutrophils and eosinophils in their defense against microorganisms and during metabolism of steroids and arachidonic acid. ROS produced by neutrophils probably participate in the carcinogenic effects of chronic inflammatory reactions. Although substantial amount of environmental carcinogens we are exposed to are present in the diet [14], our food contains also a group of molecules exerting anti-carcinogenic activity. Most of them seem to do so through antioxidative mechanisms or by activation of enzymes displaying anti-carcinogenic effects (Table 1).

More than a hundred of epidemiological studies have shown that people who consume more β -carotene (provitamin A) and have higher β -carotene levels in their blood have lower risk of cancer and particularly lung cancer [27–29]. Based on these results diet supplementation with β -carotene have been undertaken in two large clinical studies, but unexpectedly and tragically this supplementation resulted in the increased incidence of lung cancer in those smoking cigarettes [30,31]. The investigators were so shocked by these results that they initially suspected the presence of some carcinogens in β -carotene preparations used in these studies but this was found not to be the case. We still do not know the final explanation. It cannot be excluded that correlation between high β -carotene blood concentration and low cancer incidence observed in many epidemiological studies was misleading and probably high consumption of vegeta-

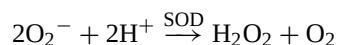
bles and fruits responsible for high β -carotene plasma levels is accompanied by intake of some other anti-carcinogenic compounds present in vegetables and fruits that have been not yet identified.

3.2. Molecules engaged in the inactivation and removal of carcinogenic substances from cells

Even if the anti-carcinogenic activity of the substances present in human diet is insufficient, our cells have at their disposal a variety of molecules able to inactivate and remove toxic and carcinogenic compounds. Against ROS, which have not been inactivated by antioxidants present in human diet we still have enzymatic defenses including [32]:

- superoxide dismutase (SOD);
- catalase;
- glutathione peroxidase.

The manganese-containing SOD2 present in mitochondria, copper- and zinc-containing SOD1 present in the cytoplasm, and SOD3 present on extracellular surfaces are very important in our defense against ROS. SOD catalyses the dismutation of superoxide radicals:



H_2O_2 is further eliminated by catalases and peroxidases [33]. There are other enzymes exerting anti-carcinogenic effects. Among these an important role is played by glutathione S-transferases (GSTs), which catalyze the conjugation of toxic and carcinogenic electrophilic molecules with glutathione, facilitating their clearance and thereby protecting cellular macromolecules including DNA from damage [34]. GSTs inactivate chemical carcinogens including polycyclic aromatic hydrocarbons found in tobacco smoke [35]. Other detoxifying enzymes, which could participate in the inactivation of carcinogens include uridine diphosphate-glucuronosyl transferase, quinone reductase, and epoxide hydrolases [36,37].

Ironically, some of the enzymes responsible for the neutralization of toxic compounds may actually activate

inactive procarcinogens into carcinogenic substances. For example, cytochrome P450 enzymes, which are engaged in the oxidative metabolism of a variety of endo- and exogenous chemicals ranging from steroids to pollutants may occasionally produce carcinogenic intermediates [38]. One of these enzymes, arylhydrocarbon hydroxylase, the activity of which is determined by the *CYP1A1* gene is responsible for the metabolism and activation of several carcinogenic substances present in the cigarette smoke, such as benzo[a]pyrene [39]. Cytochrome P450 enzyme encoded by *CYP2D6* gene, on the other hand, is responsible for the formation of carcinogens from precursors present in the cigarette smoke and perhaps in the diet as well [40]. Many P450 genes are polymorphic and individuals with certain alleles have an increased rate of carcinogen activation and an increased incidence of certain types of cancer [10,39].

The multidrug resistance proteins are membrane ABC (ATP-binding cassette) transporters engaged in the excretion of toxic or potentially toxic substances such as endogenous metabolites and xenobiotics. The multidrug resistance proteins include P-glycoprotein (P-gp) encoded by *MDR-1* gene, multidrug resistance-associated proteins (MRP1–7), the lung resistance protein, and the breast cancer resistance protein [41,42]. P-glycoprotein, the best known among these proteins, is highly expressed in the liver, kidneys, intestines, and adrenal cortex [43]. P-gp participates in the formation of blood–brain and blood–testicular barriers and is present in the placenta.

Unfortunately, the expression of multidrug resistance protein is frequently induced in tumor cells making them resistant to diverse chemotherapeutic agents. Multidrug resistance of tumor cells is thus a serious problem in the treatment of cancer and the leading cause of chemotherapy failure. It is a paradox that multidrug resistance proteins, which are expected to protect our cells against xenobiotics, including carcinogens, might fail to do so but when, as a result of their negligence, neoplastic transformation of the cell occurs, these proteins effectively “protect” tumor cells from being destroyed by the drugs used in chemotherapy.

3.3. Repair of DNA damage

If all previous defense mechanisms are breached by carcinogenic substances, they can ultimately get access to the chromatin and the damage of DNA might occur. Fortunately enough, our cells have evolved multiple DNA repair mechanisms. The importance of these mechanisms for the integrity and survival of living organisms is best illustrated by *Escherichia coli*. Up to 30% of the genes of this bacterium are engaged in DNA repair processes [44]. DNA repair mechanisms have been brilliantly compared to the immune system acting at the level of the genes [44].

Although some DNA alterations caused by simple alkylation adducts can be directly repaired by *O*⁶-methylguanine-DNA methyltransferase [45], other lesions require

participation of one of the four main DNA repair processes [46]:

- base-excision repair (BER);
- nucleotide-excision repair;
- homologous recombination (HR);
- non-homologous end-joining (NHEJ).

The significance of DNA repair in defense against cancer is underscored by the observed increase in the incidence of cancer found in most human hereditary diseases involving defects in the processing of DNA lesions [47]. Defects in the NER observed in patients with *xeroderma pigmentosum* [48] are accompanied by a marked predisposition to skin cancer development induced by sunlight exposure [6]. However, in some of the syndromes with impaired DNA repair, including *xeroderma pigmentosum*, Blooms' syndrome, and *ataxia teleangiectasia*, the increased incidence of malignancies is accompanied by immunodeficiency suggesting defective immunosurveillance [49]. Most DNA alterations are probably corrected in normal cells whereas they remain unrepaired in tumor cells leading to large number of mutations. Therefore, it has been proposed that an early event in carcinogenesis is the development of genetic instability [50] or the so called mutator phenotype [51]. Some important inducers and participants in DNA repair belong to products of tumor suppressor genes, which play an outstanding role in the defense against cancer.

3.4. Tumor suppressor genes

As mentioned above, DNA damage is probably efficiently repaired in most of the cases. If DNA alterations remain unrepaired they can induce mutations in proliferating cells only, and only mutations in a few critical genes might transform a normal cell into a tumor cell. The genes, which when mutated, have been found to contribute to transformation and tumor development, belong to two major classes, proto-oncogenes and tumor suppressor genes (anti-oncogenes). Both proto-oncogenes and tumor suppressor genes are normal cellular genes indispensable for survival. Proto-oncogenes have important roles in diverse signaling pathways, regulating cell proliferation, differentiation, and programmed cell death. They usually act as positive regulators of cell growth and are activated into oncogenes by gain-of-function mutations, translocations in the proximity of very active regulatory sequences, fusions with other genes, or they are aberrantly expressed due to amplification, increase in promoter activity, or protein stability [52,53]. As a result of gain-of-function mutations, involving point mutations, translocations, and gene amplifications, these genes, now called oncogenes, participate in neoplastic transformation.

On the other hand, tumor suppressor genes have been usually identified by their germ-line mutations that predispose affected individuals to inherited forms of cancer. Inactivation of tumor suppressor genes, due to the loss-of-function

Table 2
Examples of tumor suppressor genes [53–55]

Gene	Function of protein product
<i>RB1</i>	Transcriptional regulator, E2F1 binding
<i>p53</i>	Transcriptional regulator, induction of growth arrest and/or apoptosis
<i>APC</i>	Binding/regulation of β -catenin activity
<i>NF1</i>	Ras-GTPase activity
<i>NF2</i>	Cytoskeletal regulator
<i>p16</i>	Cyclin-dependent kinase inhibitor
<i>Wt1</i>	Transcriptional regulator
<i>VHL</i>	Regulator of protein stability and proteolysis
<i>INK4A p16^{INK4A}</i>	Cyclin-dependent kinase inhibitor for cyclin D/CDK4/6 complex
<i>p19ARF</i>	Binds MDM2 and stabilizes p53
<i>BRCA1</i>	Transcriptional regulator/DNA repair
<i>BRCA2</i>	Transcriptional regulator/DNA repair
<i>PTEN</i>	Dual specificity phosphatase
<i>PTC</i>	Transmembrane receptor for sonic hedgehog
<i>DPC4</i>	Transduction of TGF- β signals
<i>FHIT</i>	Nuclear hydrolase
<i>DCC</i>	Transmembrane receptor for netrin cell guidance molecule
<i>TSC2</i>	Cell-cycle regulator
<i>NKX3.1</i>	Homeobox protein
<i>LKB1</i>	Serine/threonine kinase
<i>E-cadherin</i>	Transmembrane cell–cell adhesion molecule
<i>MSH2</i>	DNA mismatch repair
<i>MSH6</i>	DNA mismatch repair
<i>MLH1</i>	DNA mismatch repair
<i>PMS1</i>	DNA mismatch repair
<i>PMS2</i>	DNA mismatch repair
<i>Maspin</i>	Inhibitor of tumor metastasis and tumor-induced angiogenesis

mutations or epigenetic alterations contributes to the development of cancer in a variety of tissues. Tumor suppressor genes participate in defense against cancer at almost all stages of the carcinogenic process (Table 2).

Function of the products of tumor suppressor genes includes:

- inhibition of cell proliferation,
- induction of cell senescence,
- induction of cell differentiation,
- induction of programmed cell death,
- DNA repair,
- inhibition of angiogenesis,
- interference with metastasis, and
- influence on immune surveillance.

One of the most important tumor suppressor genes is *p53* called “the guardian of the genome” for its central role in DNA repair. In normal cells under physiological conditions *p53* protein is expressed at very low levels, is highly unstable and has a low DNA binding capacity. Inheritance of a mutant allele of the *p53* tumor suppressor gene is the cause of the Li-Fraumeni syndrome characterized by an elevated risk of a variety of cancers [56]. The regulation of *p53* has been described at the level of transcription, translation, conformational changes, and covalent and non-covalent modifica-

tions [57]. One of the major regulators of *p53* is the MDM2 protein. MDM2 and JNK proteins are responsible for constitutive instability of *p53* acting as E-3-ubiquitin ligases and promoting proteasome-mediated degradation of *p53* [58].

Although initially *p53* was regarded as a sensor of DNA damage only, it has been eventually found to respond to many forms of cellular stresses including genotoxic stress, telomere attrition, hypoxia, nucleotide depletion, activation of oncogenes and growth signaling pathways [57–61]. Being a transcription factor, *p53* regulates activity of multiple genes. Over 4000 putative *p53* binding sites have been identified in human genome but the role of this protein also includes functions, which are transcriptionally independent [59].

Many forms of cellular stress including DNA damage might induce ATM, ATR, CHK1, CHK2, JNK, and DNA-PK kinases that stabilize *p53* and/or inhibit MDM2 activity [57]. ARF protein, the alternative reading frame product of the *INK4A* locus, inhibits ubiquitin ligase activity of MDM2 [57] saving *p53* protein from degradation. All the above mentioned mechanisms induced by cellular stress lead to the accumulation of a stable and transcriptionally active *p53* in the nucleus and to the activation by *p53* of a variety of genes, including that encoding p21 cyclin-dependent kinase (CDK) inhibitor, which binds cyclin-CDK4/6 complexes and causes cells to arrest in the G1 phase of the cell cycle [57,60].

In response to DNA damage, *p53* can either inhibit cell proliferation enabling cells to repair their DNA and taking direct role in BER and NER [57] or trigger apoptosis if the damage is too severe to be corrected [62]. However, the above suggested scenario is rather only speculative because the mechanisms underlying damaged cell's decision to halt divisions or to commit suicide through apoptosis have not been fully elucidated. Nevertheless, induction of apoptosis seems to be associated with higher levels of *p53* than those required for cell cycle arrest [63]. *p53* induces apoptosis by activating the expression of death receptors Fas/APO1 and DR5/KILLER [60,64–66] and expression of proapoptotic proteins Bax, NOXA, and PUMA of the Bcl-2 family [57,60].

The significance of *p53* tumor suppressor gene is underscored by the observation that in 50–55% of all human tumors *p53* mutations are found [67] and altogether mutations-induced changes in proteins of the *p53* pathway have been observed in over 80% of human tumors [68]. About 10 000 different *p53* mutations have been detected in human tumors [69]. Tumor-associated mutations in *p53* are predominantly point mutations resulting in single amino acid substitutions [70]. These mutations result not only in the abrogation of tumor suppressor activity of *p53* but they might confer on this mutated protein the ability to exert oncogenic functions by binding and inactivating the wild-type *p53* [71]. In addition to *p53*, other tumor suppressor genes can either participate in DNA repair (*BRCA1*, *BRCA2*, *MSH1*, *MSH2*, *MSH6*, *PMS1*, *PMS2*) or induce apoptosis (*PTEN*, *APC*) [54].

p53 seems also to participate in the regulation of immunosurveillance. By stimulating the expression of death receptors Fas/APO1 and DR5/KILLER [60,64–66] and death receptor ligands (FasL and TRAIL [72]) it might facilitate the elimination of neoplastic cells by T cytotoxic and natural killer (NK) cells. Moreover, p53 has also been suggested to induce expression of fractalkine, a transmembrane chemokine, which in its soluble form attracts chemotactically monocytes, T cytotoxic cells and NK cells [73].

The activity of transforming growth factor- β (TGF- β) is another example of complicated relationships between tumor suppression and immunosurveillance. TGF- β acts as an effective tumor suppressor at early stages of carcinogenesis [74] but later during tumor development it might exert oncogenic activity by promoting invasiveness and metastasis [75,76]. TGF- β ligands and their receptors are expressed in many normal cells, including lymphocytes, and also in tumor cells. In both, normal and malignant cells, with the exception of fibroblasts, TGF- β is a potent inhibitor of cell proliferation. It can also promote cell differentiation and induce apoptosis [75]. On one hand, this activity is responsible for its immunosuppressive [77], but on the other, tumor suppressor [74,76] functions. Unfortunately, during later stages of tumor development, tumor cells often manage to escape from TGF- β -mediated growth arrest due to inactivating mutations of genes encoding TGF- β receptors or signaling pathway proteins [74]. At this stage, tumor cells become refractory to TGF- β , but this cytokine still exerts immunosuppressive activity interfering with immunosurveillance and thus facilitating tumor progression.

Besides genetic defects, epigenetic changes have been found to be responsible for inactivation of tumor suppressor genes [78]. For example, hypermethylation of cytosine-guanine (CpG) islands in the promoters of *BRCA1* and *E-cadherin* genes has been observed in 13% of sporadic breast carcinomas [79] and 51% of primary gastric carcinomas [80], respectively. Increased methylation of CpG sites in regulatory regions of other tumor suppressor genes has also been found [81].

3.5. Cellular senescence

Even if p53 is inactivated, allowing cells bearing such an inactivation to proliferate despite the presence of damage in their DNA, there is another barrier limiting their growth. It has been shown that normal cells in culture do not have an unrestricted capacity to proliferate, but their growth is limited to about 60–70 doublings [82]. The cells behave as if they were having an internal clock and the capacity to count cell divisions and when the endpoint is reached an irreversible cell cycle block is imposed on them. This limited capacity to proliferate and the occurrence of growth arrest after a definite number of mitoses is probably one of the most important mechanisms responsible for the protection against immortalization of cells and development of cancer. Although cancer cells allowed to double their number about

60–70 times would be able to form a tumor of the size exceeding the human body it should also be kept in mind that many are lost due to apoptotic events [82].

Finite replicative potential of normal cells results mostly from progressive shortening of telomeres with each succeeding cell division. Telomeres form a sort of molecular caps protecting the ends of chromosomes against degradation and preventing their end-to-end fusion [83]. Therefore, to be able to proliferate unrestrictedly, tumor cells have to develop an ability to maintain their telomeres. About 90% of tumor cells succeed in doing so by acquiring the expression of telomerase, which is a reverse transcriptase capable of extending telomeric DNA [82,83]. Telomerase might be induced by the activity of *c-myc* oncogene [84]. Telomerase is also active in germline cells, stem cells, and activated lymphocytes [85]. Some tumor cells maintain their telomeres in the absence of telomerase activity by one or more mechanisms referred to as alternative lengthening of telomeres [86], for example, through recombination-based interchromosomal exchanges of DNA sequences [87].

3.6. Natural endogenous inhibitors of angiogenesis

Tumor cells that have breached all of the above described barriers can not form tumors beyond 1–2 mm in diameter. Further tumor growth depends on the development of adequate blood supply through angiogenesis. As the primary tumor grows it needs to develop a blood supply that can support its metabolic requirements, but new blood vessels can also provide an escape route by which cells can leave the primary tumor to disseminate and form metastases. Therefore, angiogenesis seems to be an essential and very important step in the growth of primary tumor, in invasiveness, metastatic process, and in the growth of metastases.

Angiogenesis is stimulated by various endogenous factors including: acidic and basic fibroblast growth factors, angiogenin, hepatocyte growth factor, interleukin 8, matrix metalloproteinases (MMP), placental growth factor, plasminogen activators, platelet-derived growth factor, transforming growth factor- β , vascular endothelial growth factors. The activity of angiogenic factors is, however, kept in check by naturally occurring inhibitors of angiogenesis (Table 3).

Tumor angiogenesis starts when the balance of angiogenic and angiostatic molecules is shifted towards the former. Angiogenesis regulators, both inducers and inhibitors, are produced by a variety of cells, including endothelial cells, stromal cells, tumor-infiltrating leukocytes, and tumor cells themselves. Products of oncogenes and tumor suppressor genes participate in the regulation of tumor angiogenesis. For example, oncogenic Ras proteins are known to effectively stimulate VEGF production [91,92] and to induce Flk-1, a high-affinity receptor for VEGF [93] and VHL tumor suppressor gene seems to be engaged in the suppression of VEGF production [94]. Angiogenesis is also controlled by p53. This protein directly stimulates the

Table 3
Some endogenous inhibitors of angiogenesis [88–90]

Angiostatin
Antithrombin III fragment
Cartilage-derived inhibitor
Endostatin
Interferon α
Interferon β
Interferon γ
Interferon inducible protein-10 (IP-10)
Interleukin-4
Interleukin-10
Interleukin-12
Interleukin-13
Interleukin-18
Maspin
Monokine induced by interferon- γ (MIG)
Platelet factor 4
Prolactin fragment
Protamine
Soluble VEGFR-1
Thrombospondin-1 and -2
Tissue inhibitors of metalloproteinases
Vascular endothelial growth factor inhibitor
Vasostatin

expression of antiangiogenic thrombospondin-1 [95] and via MDM2 targets for degradation the hypoxia inducible factor-1 α (HIF-1 α) [96], which up-regulates expression of VEGF in hypoxic tumors [97].

Tumor cells, tumor cells' fragments or tumor-associated antigens either free or bound to MHC molecules of dendritic cells (DCs) can also leave the primary tumor via lymphatic vessels to enter the regional lymph nodes. Although this mechanism should facilitate the initiation of an anti-tumor immune response, the lymphatics and regional lymph nodes often play a detrimental role. Tumor cells reaching lymph nodes via afferent lymphatics, instead of being stopped by these natural "lymph filters" proliferate there and intensively disrupt their structure. Then via efferent lymphatics they leave lymph nodes to reach the blood and disseminate to distant sites.

3.7. Natural defense against cancer at the stage of invasion and metastasis

Although even non-malignant tumor can kill the host when it grows in a sensitive organ, such as the brain, the annihilation of the host usually occurs only when tumor attains its full malignancy, which means the ability to invade the surrounding tissues and to disseminate to form metastases. It is assumed that metastases are the cause of approximately 90% of human cancer deaths [82].

Tumor invasion and metastasis consist of a series of the following steps:

- penetration of the basement membrane separating epithelial and connective tissues;
- detachment of neoplastic cells from the primary tumor;

- movement of tumor cells into blood vessels or lymphatics (intravasation);
- survival of tumor cells in the circulation;
- arrest and interaction of tumor cells with endothelial cells;
- movement of tumor cells across the blood vessel's wall (extravasation);
- migration of tumor cells into the tissue parenchyma;
- growth of tumor cells at the metastatic site.

There are certain features characteristic for human invasion and metastasis:

1. Participation of adhesion molecules at critical steps of these processes. The role of these molecules is very complex. They regulate adhesion of tumor cells to each other, to adjacent non-tumor cells (stromal cells and infiltrating leukocytes), and to the surrounding extracellular matrix. They either inhibit tumor progression or facilitate this process [98]. Therefore, the adhesion molecules that keep the cell of the given tissue together are responsible for preventing tumor invasion and metastasis and those that participate in the attachment of tumor cells to the walls of vessels, preceding their intra- or extravasation during metastatic process, are involved in the facilitation of tumor progression.
2. Dependency on angiogenesis. Only intravasation enables tumor cells to spread to distant sites. They enter blood vessels directly at tumor site or indirectly by leaving the primary tumor through lymphatics.
3. Inefficiency at each step of this process guarantees that only a small subset of tumor cells will proceed to the next one. It is estimated that less than 0.01% of circulating tumor cells will ultimately form metastatic colonies [99].
4. Organ specificity, causing malignant cells of a given origin to form metastases preferentially in selected, but not all organs. One of the explanations for organ specificity is the activity of appropriate chemokines expressed in a particular organ and the presence of receptors for these chemokines on tumor cells. However, organ specificity of metastases is probably mostly due to the presence in a target organ of growth factors stimulating the proliferation of appropriate tumor cells [100].
5. At each step, the ultimate results of the metastatic process depends on the balance of multiple endogenous inducers and inhibitors.

There are two protease systems, which play particularly important role at various stages of tumor invasion and metastasis. One of them consists of urokinase-type plasminogen activator (uPA), its receptor, plasminogen activator inhibitors (PAIs) and plasmin and another includes matrix metalloproteinases and tissue inhibitors of metalloproteinases (TIMPs). Plasmin degrades laminin, which is an important component of the extracellular matrix [101] and MMPs, which are traditionally divided into collagenases, gelatinases, and stromelysins [102], are able to effectively degrade almost all components of extracellular matrix [103]. Gelatinases

(MMP-2 and MMP-9), for example, degrade type IV collagen present like laminin in basement membranes [104] and therefore, have been implicated in tumor invasion and metastasis [104]. However, gelatinases can also facilitate the development of immune response against cancer as they enable the migration of Langerhans cells and dendritic cells, which might carry tumor-associated antigens, from skin to regional lymph nodes [105].

The degradation of the elements of extracellular matrix facilitates the invasion and metastasis by breaching basement membrane separating epithelial cells from connective tissue and basement membranes underlying endothelium in the blood vessels' walls during intra- and extravasation of tumor cells.

The urokinase-type plasminogen activator and MMPs are produced by both normal and tumor cells. In most carcinomas, enzymes degrading the extracellular matrix are produced mainly by stromal cells and tumor-infiltrating leukocytes rather than tumor cells [106].

As mentioned above, adhesion molecules can either inhibit tumor progression or enhance this process. One of the adhesion molecules, which interferes with tumor invasion and metastasis is E-cadherin involved in cell-to-cell contacts between adjacent epithelial cells. Loss of E-cadherin expression due to mutations or epigenetic changes is a common event in human cancers [107]. Although it is tempting to speculate that E-cadherin interference with tumor invasion and metastasis is due to its function in cell-to-cell adhesion, it seems more likely that its loss results in the active stimulation of tumor progression, during which β -catenin seems to play a central role. β -Catenin is at the crossroads of adherence and signaling that controls the expression of genes regulating tumor progression. β -Catenin is either free or bound to the cytoplasmic domains of E-cadherin. When β -catenin is free in the cytoplasm, it enters the nucleus where it binds transcription factors of the TCF/LEF-1 family and stimulates the expression of a number of genes including proto-oncogene *c-Myc*, *cyclin D*, *MMP-7* and *uPA* [108]. The activity of β -catenin is kept under control of E-cadherin and adenomatous polyposis coli (APC) protein, which are products of two tumor suppressor genes [108,109]. Inactivation of either E-cadherin or APC genes will result in an increase of free cytoplasmic β -catenin available for nuclear signaling [108].

Two protease systems participating in tumor invasion and metastasis are also regulated at various levels. Therefore, plasminogen activator inhibitors and tissue inhibitors of metalloproteinases may be regarded as natural inhibitors of tumor progression [99]. The role of PAIs in tumor progression may be rather complex as PAI-1 inhibits uPA interfering with tumor invasion and metastasis but, on the other hand, may protect tumor cells themselves from proteolytic degradation [110]. MMPs are regulated at the level of transcription, mRNA stability, activation from latent precursor form, and ultimately by TIMPs [102].

A substantial group of natural inhibitors that directly or indirectly regulate tumor progression are the products of tumor suppressor genes (E-cadherin, APC, DCC, maspin). Mammalian cells contain also so called metastasis-suppressor genes. These include *nm23* (*NME1*), *Kiss1*, *BrMS1*, and *maspin* [55,111]. They are particularly active in the inhibition of the metastatic process. Although the first of them, *nm23* (*NME1*) has been identified about 15 years ago [112], their mechanisms of action are still poorly understood. One of the effects of *nm23* may be to inhibit the expression of gelatinase A (MMP-2) expression [113].

There is another natural defense mechanism protecting against tumor progression, responsible for induction of apoptosis in epithelial cells, which lost cell-to-matrix anchorage [114,115]. The mechanism of this detachment-induced apoptosis, called anoikis has not been fully elucidated but various molecules including integrins, Bcl-2 family proteins, p53, caspases, β -catenin, and caveolin-1, have been implicated to participate in its regulation [114–120]. Somehow anoikis is also evaded by tumor cells during neoplastic progression.

As mentioned above, only a small fraction of tumor cells manage to metastasize to distant organs. Therefore, the efficacy of natural anti-tumor defense mechanisms operating at the stage of tumor invasion and metastasis is enormous. It seems, however, that substantial part of defense at these late stages of neoplastic progression involves the activity of various immune mechanisms.

4. Immune mechanisms of protection against cancer

4.1. The concept of tumor immunosurveillance

The tumor immunosurveillance hypothesis, first raised by Paul Ehrlich in 1909 and then refined by Burnet and Thomas, postulated that the immune system constantly surveys the newly developing tumors and, as long as it is effective, prevents the development of neoplastic disease. It was assumed that clinically evident tumors represent exceptions that slipped through the immunological net.

The increased incidence of tumors in immunosuppressed recipients following organ transplantation, and in patients with inborn or acquired defects of the immune system is frequently regarded as an incontrovertible evidence for this belief. This hypothesis is also supported by the observations that reduced immunosuppression in transplant recipients and partial restoration of the immune response in AIDS patients during highly active antiretroviral treatment (HAART) can induce tumor remission [121]. However, immunosuppressed patients are not at an increased risk of developing the most frequent epithelial malignancies such as breast, lung, colon or prostate cancers. Intriguingly, the occurrence of some epithelial cancers is even reduced in AIDS patients [122]. Closer examination of tumors that developed in patients with defects in the immune function revealed that most were asso-

ciated with viral infections. Moreover, neither the frequency of spontaneous tumors nor shortened latency of chemically induced cancers in athymic nude mice was observed, except for some lymphoreticular tumors [123]. Although nude mice do not have thymus and thus lack functional T-cell immunity and T-cell dependent humoral immunity, they still have $\gamma\delta$ T cells and an apparently intact innate immunity that might be engaged in the protection against cancer. Finally, there is no greater incidence of tumors in the so called immunoprivileged sites (such as brain, thymus, anterior chamber of the eye) that at least to some degree are excluded from the immunosurveillance.

Overall, although there is no irrefutable evidence for the existence of the effective immunosurveillance against epithelial cancers in humans it appears that the “immune watchdog” might play a role in virally induced or virus-associated malignancies which constitute about 15% of all tumor cases in humans. The concept of immunosurveillance was recently revived by the observations that spontaneous as well as chemically induced tumors develop more frequently in genetically immunocompromised (knock-out) animals.

4.2. Innate immune mechanisms in immunosurveillance

4.2.1. Macrophages and neutrophils

Macrophages and neutrophils can be regarded as both heroes and villains in the context of tumor development. On the one hand, these cells are capable of phagocytosis of and antibody-dependent cellular cytotoxicity (ADCC) towards tumor cells, and secretion of tumor-growth inhibitory cytokines. On the other hand, activated neutrophils can induce transformation of normal cells in vitro [124,125] and epidemiological studies established a close association between chronic inflammation and tumor incidence (Table 4).

What might be the mechanism of the carcinogenic effects of chronic inflammation? It is taken for granted that increased release from activated phagocytes of mutagenic reactive oxygen species or nitric oxide are responsible for the DNA damage and neoplastic transformation. However,

it should also be kept in mind that phagocytic cells have evolved perfect mechanisms of limiting the extracellular spill of reactive oxygen species. ROS are mainly released to the phagocytic vacuole, and the minute leakage is efficiently scavenged by extracellular enzymes (as discussed earlier). Moreover, the extremely short half-life and short range of diffusion of ROS is limiting their mutagenic capabilities. Surprisingly, administration of the radical scavenging anti-oxidant β -carotene does not reduce, but actually increases the risk of cancer in humans [30,31].

Despite the fact that DNA synthesis is a precise process, errors do occur, and can lead to mutations. Cell divisions are necessary to convert these potentially transient changes in the DNA into fixed lesions (mutations). It is therefore intuitive that an increased turnover rate, impaired differentiation or apoptosis due to the action of inflammation-associated cytokines or growth factors might also result in acquisition of mutant phenotypes. There is a number of studies that confirm the tumor growth-promoting effects of cytokines:

- prolonged secretion of IL-15 enhances the formation of lymphomas in mice [128],
- M-CSF facilitates development of breast carcinomas [129],
- TNF is essential for the elicitation of skin carcinomas by chemical promoters [130,131],
- IL-6 is necessary for the development of carcinogen-induced lymphomas [132], and
- macrophage inhibitory factor (MIF) antagonizes the function of p53 [133].

Paradoxically, most of these cytokines, alone or in combination have also been effective in the treatment of experimental tumors in mice [134–137].

Observations in humans and in animal models indicate that prostaglandins, which are involved in the development of inflammatory processes, can contribute to the development of cancer. Many cancer types as well as inflammatory cells, endothelial cells and fibroblasts express inducible cyclooxygenase (COX-2). Prostaglandins, such as PGE₂, can stimulate cell proliferation, induce synthesis of growth factors, induce angiogenesis, inhibit apoptosis of tumor cells by increasing expression of antiapoptotic Bcl-2 protein, stimulate cell signaling through peroxisome proliferator-activated receptor delta (PPAR δ) that regulates the expression of proliferation-associated genes, and exerts immunosuppressive effects [127,138,139]. Moreover, COX-2-catalyzed synthesis of prostaglandins is accompanied by the formation of DNA-reactive by-products with mutagenic potential, such as malonyldialdehyde [127,140]. Overexpressed COX-2 enzymes can also catalyze the oxidative metabolism of xenobiotics that leads to the formation of genotoxic mutagens [127,140].

Chronic inflammation can also precipitate epigenetic changes in normal cells. Unsaturated aldehydes derived from eicosanoid biosynthesis or lipid peroxidation (malonyldialdehyde, 4-OH-nonenal) or unsaturated ketones

Table 4
Examples of chronic inflammation and an increased cancer incidence [126,127]

Inflammatory stimulus	Tumor
Hepatitis B and C viruses	Hepatocellular carcinoma
Human herpesvirus type 8 (KSHV)	Kaposi's sarcoma
<i>H. pylori</i>	Gastric carcinoma
Papilloma virus	Cervical carcinoma
Schistosomiasis	Bladder carcinoma
Reflux esophagitis, Barrett's esophagus	Esophageal carcinoma
Ulcerative colitis	Colorectal carcinoma
Cholangitis	Cholangiocarcinoma
Asthma	Lung carcinoma
Asbestosis, silicosis	Mesothelioma, lung carcinomas

derived from eicosanoid metabolism have all been shown to impair the function of p53, a “guardian of the genome” [126]. Similar effects have been ascribed to nitric oxide, which together with its metabolites such as peroxynitrite and NO₂ can cause DNA damage and has been shown to be mutagenic [141].

Phagocytic cells may also contribute to more advanced stages of tumor progression. It has been known for over a century that malignant growth is frequently accompanied by a dense inflammatory infiltrate. The still unanswered question is whether these cells are involved in promoting progression or in the destruction of tumors. The protective role of inflammatory cells in the development of tumors has been demonstrated in a series of studies. However, the intensity of the leukocyte infiltrate still remains of unproved prognostic significance [142]. Although macrophages and neutrophils are capable of phagocytosis and ADCC towards tumor cells and can secrete a number of cytokines with direct (TNF, TRAIL) and indirect (IL-12, IL-18) anti-tumor activity, there is an equal number of studies that implicate these cells in the neoplastic progression. For example, inhibition of macrophage infiltration correlated with the inability of the tumor to grow in vivo [143], but on the other hand, there is evidence that expression of macrophage activating cytokines by transfected tumor cells resulted in the retardation of tumor growth [144]. Cytokine activated neutrophils demonstrate significant anti-tumor effects both in vitro and as inflammatory infiltrate in vivo [145], but these cells have also been implicated in the T-cell dysfunction observed in tumor-bearing mice [146]. Macrophage and neutrophil activating cytokines demonstrate indisputable anti-tumor effects in numerous tumors [145,147–149] but the same cytokines can also promote tumor growth in other tumor models [150,151]. Moreover, IL-8 transfected tumors with prominent neutrophil infiltration acquire a rapid genetic instability that correlates with acquired mutations in the investigated locus [152]. Other cells, such as mast cells can also be found among tumor-infiltrating leukocytes. These cells, together with tumor-associated macrophages, can be a source of matrix metalloproteinases that, through degradation of the extracellular matrix, contribute to the mobilization of tumor-associated fibroblast and endothelial cells resulting in the promotion of angiogenesis [153].

Tumors can also be infiltrated by eosinophils, which release peroxidase and major basic protein, powerful cytotoxins used in the elimination of parasites and also damaging to normal tissues. Although eosinophil infiltrates are rela-

tively infrequent, their presence correlates with better prognosis [154].

Tumors can acquire more aggressive growth by producing chemokines that attract inflammatory cells. The “tumor-friendly” mediators released by these cells may act back and stimulate tumor cells proliferation (PDGF) or influence angiogenesis (TNF), invasion and metastasis (numerous chemokines). Moreover, several mediators (PGE₂, TGF-β, VEGF) are capable of preventing the development of specific immune response against tumors. Therefore, there is no convincing evidence to support the concept that macrophages or neutrophils play a significant role in tumor immunosurveillance. A more practical issue is whether it is possible to exploit the cytotoxic armamentarium of these cells to more effective tumor destruction. Experimental studies show that adoptive transfer of activated macrophages results in significant anti-tumor effects and the effectiveness of this approach is already tested in clinical trials [155]. Moreover, antigen-specific immune responses are always accompanied by the effector mechanisms of innate immunity.

4.2.2. Complement

Activation and subsequent deposition of complement components in tumor tissue has been demonstrated in numerous studies [156–159]. However, the role of complement in immune surveillance against cancer has not been unequivocally demonstrated. Tumor-bound antibodies usually mediate its activation, in the context of an anti-tumor response. Therefore, this system seems to be operative only when the effective humoral response has developed.

Despite numerous observations that tumor cells are protected from complement-mediated attack by cell-surface inhibitors, such as CD35, CD46, CD55 or CD59 (Table 5), there is no evidence that these protective mechanisms are of any importance in the neoplastic progression. All of these protective mechanisms are also employed by normal nucleated cells to protect them against spontaneous complement activation. Apparently, the mechanisms of complement resistance might be important in protecting tumor cells from complement activation mediated by therapeutically used monoclonal antibodies.

4.2.3. NK cells

In contrast to T and B lymphocytes that require a proliferation phase that selects a population of effectors, NK

Table 5
Complement regulatory proteins and their mechanism of action

Complement regulatory protein	Mechanism of action	Upregulation in human cancers
CD35 (complement receptor type 1; CR1)	Cofactor for factor I-mediated cleavage of C3b and C4b	Yes
CD46 (membrane cofactor protein; MCP)	Prevents the formation of C3/C5 convertases	Yes
CD55 (decay accelerating factor; DAF)	Inhibits formation and dissociates preformed C3/C5 convertases	Yes
CD59 (protectin)	Inhibits C9 polymerization and formation of the membrane attacking complex	Yes

cells are charging immediately on finding their targets. These cells do not require sophisticated gene rearrangement machinery to assemble specific receptors but nonetheless exhibit a unique capacity to discriminate correct target cells. Although in many cases NK cells activity against tumor cells correlates with decreased levels of MHC molecules, the phenomenon referred to as “missing self” hypothesis, the most recent observations indicate that insufficient expression of MHC molecules might not always be necessary for effective target cells killing. NK cells were shown to kill a number of tumor cells despite significant levels of MHC class I molecules on their surface [160–163]. This implies that the activation of NK cells is not only a result of a loss of MHC class I alleles but also results from the direct recognition of target cell structures. The identification of activating or triggering receptors of NK cells and their ligands in target cells helped in elucidating a number of important unresolved issues regarding the control of NK cells activity. It now appears that NK cells have evolved not only to cooperate with adaptive immunity (e.g. by expressing CD16, a FcγR for IgG and secretion of cytokines that regulate the function of T cells) but they also contain a whole armamentarium for rapid detection and elimination of potentially “dangerous” cells.

4.2.3.1. Inhibitory NK cell receptors for MHC class I molecules. Several types of inhibitory receptors have been identified in human NK cells that detect the presence of all types of class I MHC molecules. These receptors include the CD94: NKG2A heterodimers, the killer-cell immunoglobulin-like/inhibitory receptor (KIR) family, and some members of the leukocyte immunoglobulin-like receptor/immunoglobulin-like transcript (LIR/ILT) family that represent non-redundant but complementary systems for the detection of MHC class I molecules.

The CD94: NKG2A heterodimers recognize sequence elements conserved in the signal peptides of most MHC class I molecules, presented in association with HLA-E molecules (which cannot present their own signal peptide). On the other hand, KIR receptors (and their murine Ly49 counterparts) directly recognize polymorphic determinants of HLA-A, -B or -C molecules, and themselves represent a diversity of molecules that is nearly comparable to the MHC class I molecules. The LIR1/ILT2 molecules are involved in broad MHC class I recognition, while LIR2/ILT4 appears to recognize HLA-F.

Downregulation of antigen expression is a relatively simple mechanism of avoiding a specific immune response, especially in genetically unstable tumor cells. No wonder that tumors surveilled by cytotoxic T cells are frequently composed of cells with defective antigen presentation (for the mechanisms see below). Hopefully, our immune system “trained” by pathogens is at least partly prepared for such “cunning tricks” of tumor cells.

4.2.3.2. Triggering NK cell receptors. The activation of NK cells results from the concerted action of triggering receptors as well as cytokines, co-stimulatory and adhesion molecules. It appears that triggering receptors can initiate NK cell activation and target cell lysis, provided that NK cells are not turned off by the inhibitory receptors.

The natural cytotoxicity receptors (NCR) responsible for triggering of NK cells cytotoxicity include NKp30, NKp44 and NKp46. NKp30 and NKp46 are constitutively expressed by all NK cells, whereas NKp44 is inducible by cytokines. Although all have been implicated in NK cell-mediated recognition and killing of tumor cells, no ligands have so far been identified. It seems that simultaneous activation of cytotoxicity receptors is required for the most effective tumor cells’ killing [164].

Next to classical natural cytotoxicity receptors responsible for triggering cytotoxicity, there are also MHC class I-specific receptors in each family of inhibitory receptors [165,166]. Activating receptors in the KIR family include molecules with a short cytoplasmic tail lacking activating ITAM sequence. This KIR subfamily includes four molecules: KIR2DS1, KIR2DS2, KIR2DS3 and KIR2DS4. Although these molecules seem to recognize MHC class I molecules, analogously to inhibitory KIR receptors, it seems that stimulatory and inhibitory receptors of the same class I specificity are not expressed by the same NK cell. The NKG2 receptor family contains stimulatory NKG2C and NKG2E molecules that heterodimerize with CD94 [165,166].

Another natural cytotoxicity receptor, NKG2D, is distantly related to NKG2A and does not associate with CD94. It homodimerizes and for full activity requires association with an adaptor protein DAP10 (or KAP10). The ligands for NKG2D are non-classical MHC class I-related MICA and MICB molecules encoded by genes within the human MHC [167–169]. Both are stress-inducible and mostly expressed in tumor cells of epithelial origin [170]. Importantly, NK cells are able to kill tumors expressing MICA proteins despite the presence of MHC class I molecules on these targets. Analogously, murine NKG2D binds two other loosely related to MHC class I molecules: Rae-1 and H60 [167]. Both human and murine NKG2D ligands display a selectively regulated expression pattern and are found on tumor cells but not in normal adult tissues. These molecules might therefore be regarded as “tumor-specific” markers for recognition by NK cells. Although human Rae-1 and H60 equivalents have recently been identified [171] there are still no evidence that they might function in an analogous manner as ligands for human NK cells. The identification of stress-inducible ligands capable of triggering NK cells cytotoxicity has revived the interest in the immune surveillance hypothesis.

4.2.3.3. The role of NK cells in the immune surveillance. While the role of NK cells in the natural protection against tumors in humans is still a matter of debate, the animal studies have unequivocally shown that natural

cytotoxicity is an effective tumor immunosurveillance phenomenon. Beige mice with a minimal NK cell activity, animals treated with anti-asialo GM1 or anti-NK1.1 antibodies or simply treated with cyclophosphamide developed an increased number of spontaneous and carcinogen-induced tumors. Accordingly, studies with mice transgenic for Ly49A that have defective natural killing as well as selective deficiency in NK1.1⁺CD3⁻ cells revealed a critical role for NK cells in suppressing the outgrowth and metastasis of tumor cells [172].

Circumstantial evidence for the protective role of NK cells is provided by observations of patients with Chediak–Higashi syndrome, X-linked lymphoproliferative syndrome, or renal transplant recipients treated with immunosuppressive drugs [173,174]. Although all have a low NK cells activity the increase in the incidence of just few cancer types is observed.

Although in most studies, the natural cytotoxicity was reduced in patients with cancer of various origin [175], with even further reduction in more advanced disease [175], it has not been resolved whether the low activity precedes the development of malignancy or is secondary to the overt malignant process. It must be emphasized, however, that in some studies there was no reduction in natural cytotoxicity in patients with cancers, irrespective of the stage of disease [175]. Moreover, studies of blood donors with a decreased NK activity did not seem to reveal an increased cancer incidence [176].

The difficulty in the interpretation of studies on NK cells activity stems from the wide biologic variations in the basal activity of these cells among healthy controls [175]. Moreover, cancer-associated malnourishment and concomitant therapies should be taken into consideration while interpreting the results of natural cytotoxicity measurements in patients with already diagnosed cancer.

In order to establish reliable correlation between cancer incidence and natural cytotoxicity levels, large groups of patients should be evaluated in prospective studies. Curiously, in one such study the NK cell function was found to be significantly higher in women with risk factors for breast cancer development than in healthy controls [177]. Regrettably, no follow-up on the incidence of breast cancer in these women has so far been published. In a prospective study of Japanese population, an 11-year follow-up indicated that medium and high natural cytotoxicity of peripheral blood lymphocytes is associated with reduced cancer risk, and low natural cytotoxicity correlates with increased cancer incidence [178]. In another study, patients with hepatic cirrhosis had low NK activity and an increased incidence of hepatocellular carcinoma [179].

Another important issue is whether NK cells are able to influence the course of the neoplastic disease. In this regard, some prospective studies revealed that reduced NK cell function correlates with poor prognosis (higher risk of development of regional and distant metastases and increased mortality) and increased risk of recurrence following surgery

[180–183]. However, this conclusion was not reached in other studies [184,185].

4.2.4. NKT cells

NKT cells are CD1d-dependent, MHC-independent lymphocytes, with a heavily biased TCR repertoire and NK1.1 molecule, usually described as NK1.1⁺TCR $\alpha\beta$ ⁺ cells [186]. In mice, most express an invariant TCR α chain (V α 14J α 18) and either V β 8.2, V β 2 or V β 7 chain, which are homologues to V α 24 α 15 and β 11 chains, typical for human NKT cells [187]. They also express activating and with inhibitory receptors typical for NK cells [188]. Upon stimulation, NKT cells secrete immunoregulatory cytokines characteristic of both Th1 and Th2 responses, including IL-4, IL-10, IL-13, TNF and IFN- γ [189,190]. These cells also possess a powerful armamentarium of death-inducing effector molecules such as perforin and TRAIL. They initiate effective anti-tumor responses when stimulated with exogenous factors such as low-dose IL-12 or α -galactosylceramide (α -GalCer). NKT cells were also shown to play a critical role in the protection from carcinogen-induced tumor development in mice [191]. Their anti-tumor activity results mainly from perforin-dependent cytotoxicity, IFN- γ production and the augmentation of dendritic cell, NK and CD8⁺ T cells function [192]. In apparent contrast, NKT cells have also been shown to suppress tumor-specific, CTL-mediated tumor rejection in an IL-13-dependent manner [193], and to suppress adaptive immune response against UV-induced skin cancers [194].

4.3. The role of cytokines in the immunosurveillance

Cytokines participate in the regulation of every aspect of the immune response. Their role is sometimes so pleiotropic that it is virtually impossible to pinpoint a physiological role and it is even more difficult to establish their influence on the natural mechanism of defense against tumors. The potential anti-tumor effects of cytokines have been established in numerous studies during which a given cytokine has been administered exogenously. Although some cytokines are capable of producing anti-tumor effects in some models they can also accelerate tumor development in others. The list of cytokines that exert anti-tumor effects is very long. However, not all have been demonstrated to play a role in tumor immunosurveillance. The definite information on the role of cytokines in tumor development can be obtained during studies with knock-out animals.

The advent of targeted gene mutation has revolutionized the studies on the immunosurveillance against spontaneous and carcinogen-induced tumors. Co-incident mutation of genes encoding p53 and perforin demonstrated a critical role of the latter in the protection against spontaneous lymphoma development [195]. A similar role can be contributed to the Fas–FasL interaction in the control of plasmacytoid

tumors development [196]. Similarly, knocking-out of numerous genes encoding cytokines revealed a number of important immune mechanisms that seem to control carcinogenesis in mice (see below). The inherent question remains as to the role of all these phenomena in cancer development in humans.

4.3.1. Type I interferons

IFN- α -based therapy has been used successfully for the treatment of several malignancies, with greater activity in premalignancy and in locally advanced lesions than in metastatic diseases [197,198]. Studies in squamous cell carcinoma of the skin revealed that one of the initial events in the carcinogenesis is the suppression of the type I interferon signaling proteins and hyporesponsiveness to endogenous IFN [199].

4.3.2. The role of IFN- γ in the protection from tumor development

IFN- γ regulates tumor growth through either direct or indirect effects by modulating the immune response. It exerts cytostatic or cytotoxic effects towards tumor cells, induces the expression of chemokines IP10 and MIG that inhibit angiogenesis, upregulates the expression of MHC class I and II molecules and is one of the most potent activators of macrophages [200]. Initial studies revealed the role of IFN- γ in promoting rejection of transplantable tumors but did not address the problem whether this cytokine prevents primary tumor development [201,202]. Mice lacking either IFN- γ , its receptor (IFNGR1 $^{-/-}$) or the most critical signal transducing element (STAT1 $^{-/-}$) were more susceptible to the development of carcinogen-induced sarcomas than their normal counterparts [203–205]. In knock-out animals, tumors developed with shorter latency and at lower doses of carcinogens than in controls. Similarly, mice insensitive to IFN- γ crossed with p53 $^{-/-}$ mice developed spontaneous tumors earlier than mice lacking p53 alone [203]. Moreover, whereas p53 $^{-/-}$ mice developed mainly lymphomas the double knock-outs additionally formed non-lymphoid tumors. Similarly, mice without both STAT1 and genes responsible for the rearrangement of specific antigen recognition receptors (RAG2 $^{-/-}$) spontaneously developed adenocarcinomas of the colon, lung and breast at higher frequency than single knock-out animals [205]. Finally, IFN- γ knock-out mice were shown to spontaneously develop lymphomas, thymomas and even lung adenocarcinomas [206]. Although IFN- γ can participate both in the innate and adaptive immunity, the overwhelming data indicate that the tumor-protecting role can be attributed to NK and NKT cells. Altogether these studies reveal that in mice IFN- γ plays an important role in the natural protection from tumor development. Although in some tumor models it was found to promote metastasis [207], the role of this cytokine in human immunosurveillance against tumors has not been yet elucidated.

4.3.3. TNF superfamily and tumor development

A long and a fascinating history led to the discovery and naming of the tumor necrosis factor. Cloning of the gene and production of a recombinant protein rose great hopes for its therapeutic activity. These were unfortunately shortly dispelled when unacceptable toxicity led to its withdrawal from clinical studies [208]. The anti-tumor effects of TNF can be attributed to both direct cytotoxic/proapoptotic effects on tumor cells and to its influence on the immune response, tumor angiogenesis, lipid metabolism and procoagulant properties.

Mice deficient in TNF are resistant to carcinogen-induced skin tumor development [130,131] and TNF receptor type I knock-out mice do not develop liver cancers [209]. Together with the fact that TNF is directly transforming for 3T3 fibroblasts in vitro [210] one could wonder whether this cytokine is indeed a tumor necrosis or a “tumor promoting factor”. TNF is expressed in a range of human tumors and is usually associated with poor prognosis [211]. TNF is an autocrine growth and survival factor for several tumor cells [212], besides displaying angiostatic effects it also induces the secretion of angiogenic factors [213], influences tissue remodeling by induction of matrix metalloproteinases [214], increases metastatic potential of tumor cell types [215], and is responsible for the resistance to some chemotherapeutics [216]. Therefore, the action of TNF in cancer is at least paradoxical. Undeniably, TNF at supra-physiological doses exerts impressive anti-tumor activity, but at the cost of systemic toxicity. Chronic, low-dose production of endogenous TNF contributes to the neoplastic progression. There is still hope that we could sometime sort out the good from the bad and use the best of its anti-tumor effects for the therapeutic purposes.

TNF-related apoptosis-inducing ligand (TRAIL) has been shown to kill transformed, but not most normal cells, in vitro. The loss of TRAIL receptors expression on tumor cells (especially in metastatic lesions) supports the hypothesis that this effector molecule might also play a role in an endogenous protection from tumor progression [217,218]. TRAIL is a key effector molecule in the anti-tumor activity of interferon-stimulated macrophages and T cells. Moreover, TRAIL plays a critical role in the NK cell-mediated surveillance against tumor development in mice treated with a chemical carcinogen methylcholantrene or in mice with mutant p53 [219,220]. TRAIL knock-out mice are significantly more susceptible to experimental and spontaneous tumor metastases and are prone to an accelerated progression of transplanted tumors [219,221]. However, in contrast to STAT-1 $^{-/-}$, IFN- γ $^{-/-}$ or RAG $^{-/-}$ mice, TRAIL-mutant animals did not develop spontaneous tumors. Interestingly, in one experimental tumor model, neutralization of TRAIL led to a significantly delayed growth of mastocytoma [222].

4.3.4. IL-12

Interleukin-12 is a cytokine that regulates the transition from innate to adaptive immunity. Produced mainly by monocytes/macrophages and dendritic cells, it induces

secretion of IFN- γ and other cytokines from T, NK and NKT cells, regulates the proliferation and cytolytic activity of these cells and influences the secretion of complement-fixing antibodies from B cells (reviewed in [223]). IL-12 is a central regulator of Th1 cells development and exerts strong anti-tumor effects when administered to mice. When administered exogenously, it prevents mammary tumor development in transgenic mice that express *HER-2/neu* oncogene under the transcriptional control of mouse mammary tumor virus [224] and suppresses chemical carcinogenesis [225]. Knock-out mice that lack one of the IL-12 subunits are more susceptible to 3-methylcholantere-induced carcinogenesis [191]. Tumors develop faster and in about twice as many knock-out mice as compared with wild type animals.

4.4. Adaptive immune mechanisms in immunosurveillance

The initial tumor transplantation experiments in mice revealed that these animals could mount protective immune responses against tumors. However, in these early experiments tumors were transplanted between outbred strains of mice and the rejection resulted from the recognition of allogeneic MHC molecules [226]. With the development of inbred strains of mice it became possible to study the protective effects of the adaptive immune response against tumors. The first unequivocal demonstration that tumor cells express antigens capable of eliciting specific and protective immunity came from studies in the 1950s with murine tumors induced by chemical carcinogens or radiation [227]. Surgical resection of a primary carcinogen-induced tumor resulted in the rejection of a subsequent challenge with the same tumor. However, transplantation of this tumor into other genetically identical mice was followed by tumor growth that eventually killed the hosts. Continuation of these studies revealed a remarkable heterogeneity of tumor antigens expressed in carcinogen-induced tumors with each individual tumor expressing a unique set of antigens not shared even by those tumors that were induced in exactly the same manner [228]. The rejection of the transplanted tumors displayed the cardinal features of adaptive immunity, including memory and specificity. Since these tumor antigens were detected by the rejection of transplanted tumor cells and were different for every tumor studied, they are referred to as tumor-specific transplantation antigens (TSTA).

Most of these studies were done with tumors that were induced by administration of high doses of chemical carcinogens, UV light or irradiation. Normal mice transplanted with such tumors face cancer cells that potentially contain hundreds of mutated peptides (potential tumor antigens) and developed in the absence of any immune selective pressures. TSTA might therefore be a laboratory artifact. Nonetheless, these observations sparked further interest in studies on tumor-associated antigens and revealed that tumors can be immunogenic.

Spontaneous murine tumors were repeatedly shown to be non-immunogenic. Similarly, humans are infrequently

exposed to high and, at the same time, immunosuppressive doses of carcinogens. Most tumors develop due to the chronic exposure and to many different cancer-inducing agents and most genetic changes in cancer are immunologically silent. Therefore, it is unlikely that neoplastic transformation will often lead to the development of immunogenic tumor cells. Transformed cells might initially contain too little tumor antigens to mount an effective anti-tumor response, a phenomenon sometimes referred to as immune ignorance. With time there is a gradual increase in the number of tumor cells and in the amount of tumor antigens. However, this situation resembles allergen immunotherapy that is essentially based on the administration of initially low but increasing doses of particular hypersensitivity causing allergens. This situation leads to development of immune tolerance rather than immunity.

Since tumor transplantation experiments are clearly not possible in humans no evidence for the existence of TSTAs in man exists. Nonetheless, tumor antigens including occasionally tumor-specific antigens have been described in human tumors and the involvement of adaptive immunosurveillance against tumors is supported by the observations of spontaneous remissions with immune-background, effective immunotherapy in some patients and from the existence of a number of immune-evasion strategies of tumors (see below).

4.4.1. Tumor antigens

Treatment of non-immunogenic spontaneous murine tumors with mutagens resulted in the generation of tumor variants that were rejected in transplantation experiments. The resultant Tum⁺ variants are cells that are not tumourigenic due to increased immunogenicity [228]. Similarly, serial in vitro co-cultures of immunogenic (regressing after transplantation) tumor cells with cytotoxic T cell clones results in the selection of antigen loss variants that are highly tumourigenic after transplantation [229].

Molecular cloning of murine tumor antigens responsible for regressions of Tum⁺ variants of mastocytomas was followed by the identification of dozens of human tumor-associated antigens. These antigens are now classified into several different categories (Table 6) but only some of them are truly unique and can be regarded as tumor specific. Most tumor-specific antigens are products of mutated genes that either contribute to or are irrelevant to tumor

Table 6
Human tumor antigens [230,231]

Tumor antigen category	Examples
Melanocyte differentiation antigens	Tyrosinase, gp100, MART-1/MelanA, TRP-1, TRP-2
Tumor-specific antigens	CDK4, MUM-1, β -catenin, caspase-8
Cancer testis antigens	MAGE-1 and 2, BAGE, GAGE, RAGE, NY-ESO-1
Promiscuous antigens	p15, PRAME, SART-1, DAM, CAMEL, HER2/neu, MUC-1

development. Also virus-induced tumors express specific antigens, but these usually correspond to viral proteins.

4.4.2. Spontaneous remissions of human cancer

Spontaneous regression of malignant tumors in humans is observed rarely and is reported in virtually all types of cancer, although the greatest number of cases involves patients with neuroblastoma, renal cell carcinoma, malignant melanoma, lymphomas and leukemias. Although multiple mechanisms seem to be implicated in spontaneous tumor regression including induction of differentiation or apoptosis of tumor cells, inhibition of tumor angiogenesis, hormonal influences, and the impact of psychological factors, and epigenetic mechanisms [232] the adaptive immunity is usually regarded as the most important.

The immunosurveillance is also presumed to be responsible for some cases of tumor dormancy during which tumors or their metastases remain clinically asymptomatic or undetectable and do not progress for months or even years after their first appearance [233]. Tumor dormancy in breast cancer patients was found to last up to 20–25 years [234].

4.4.3. Validation of tumor immunosurveillance hypothesis in knock-out animals

The identification of a tumor antigen requires it to be recognized by human T or B cells. Although in many patients with tumors antibodies and T cells able to recognize tumor-associated antigens have been identified [235], there is no direct proof that they play a role in the control of the tumor growth. Nonetheless, studies in genetically modified animals confirm the significant role of adaptive immunity in the control of neoplastic progression. For example, mice doubly deficient for the recombination-activating gene 2 ($RAG2^{-/-}$) and the gene encoding transcription factor mediating signaling by interferon receptors ($STAT-1^{-/-}$), have an increased risk of development of mammary and gastrointestinal carcinomas in a methylocholanthrene (MCA)-induced tumor models [205]. In perforin deficient mice, an increased risk of spontaneous lymphoma development is observed. Accelerated lymphomagenesis in these mice can be attributed to defective $CD8^{+}$ T cell cytotoxicity [195], although NK cells also contribute to the control of metastases in these mice [236]. Finally, mice lacking $\gamma\delta$ T cells were shown to be highly susceptible to cutaneous carcinogenesis [237].

4.4.4. Tumor escape mechanisms

In the majority of tumors in humans, cancer cells develop various strategies to evade the immune system and to inhibit anti-tumor response [238,239]. Immune escape mechanisms can operate during both induction of the immune response and in the effector phase.

4.4.4.1. Failure in induction of the anti-tumor response.

In immunocompetent host, tumor cells adapt to the B and T cell recognizing system through selective development

of either weakly immunogenic or even non-immunogenic clones. According to the two-signal hypothesis, the presence of costimulatory molecules is necessary, apart from an intact TCR-mediated recognizing system, to induce effective immune response [240]. Lack of costimulatory molecules on tumor cells accompanied by the absence of appropriate “danger” signals enabling effective function of antigen presenting cells (APC), including dendritic cells, induce long-term tolerance instead of activation of the anti-tumor response [241]. In patients with metastatic melanoma, Th2 pattern of response and production of immunosuppressive cytokines such as IL-10 in situ were associated with poor function of tumor-derived DCs and progression of metastases.

Inadequate induction may result also from low expression of tumor antigens or from the inability of their expression [242]. In many tumor types, defective antigen processing has been reported [239]. The defects may result from down-regulation of the proteasome subunits LMP2 or LMP7 as well as from mutation and ineffective function of molecules responsible for the trafficking of proteasome-degraded proteins into endoplasmic reticulum, such as transporter associated with antigenic processing (TAP) [239,243].

Insufficient induction of the anti-tumor response may also result frequently from down-regulation or loss of MHC molecules on tumor cells [242].

4.4.4.2. Suppression of the effector phase. In some cancer patients, the presence of specific $CD8^{+}$ and $CD4^{+}$ T cells in the blood or tumor tissues and antibodies recognizing tumor antigens has been reported [235]. However, as a rule, no apparent clinically significant anti-tumor effect is observed in cancer patients [244]. There are several reasons of this “immune insufficiency”. Usually, in clinically advanced tumors, tumor growth kinetics exceeds the rate of elimination of tumor cells by immunological mechanisms. Next, tumor cells are heterogenic and even within the same tumor nodule cells differ in the phenotype and expression of tumor antigens. Epitopes from the same tumor antigens are variously exposed and effective immune response may be directed only against a fraction of the total population of tumor cells [242,245]. Finally, at the tumor level, complex interactions between tumor cells, microenvironment and the immune system take place, altogether leading to the local immunosuppression [238,244]. The mechanisms responsible for this phenomenon include:

- secretion by tumor cells (and also by cells of the immune system) of generally immunosuppressive mediators: TGF- β , IL-10, PGE₂, VEGF [235,238]. The latter cytokine, apart from antiangiogenic properties, inhibits development of dendritic cells;
- expression of apoptosis-inducing molecules, such as FasL (CD95L) or RCAS1, on tumor cells, targeted at tumor-infiltrating lymphocytes (TIL) [246,247];

- production by tumor cells of molecules aimed at blocking effector T cells, e.g. DcR3—a decoy receptor that neutralizes FasL [248];
- decreased expression of the death receptor Fas (CD95) or expression of inhibitors of signals transmitted by death receptors in some tumor cells, rendering these cells resistant to T-cell attack [239,249].
- increased expression in tumor cells of membrane-bound complement regulatory proteins (e.g. CD46, CD55, and CD59) responsible for inactivation of some components of the complement system [250];
- signaling defects in T cells from cancer patients including downregulation or loss of ζ chain associated with T-cell receptor/CD3 complex [251].

References

- [1] M. Burnet, *Br. J. Cancer* 1 (1965) 338–342.
- [2] R.T. Prehn, *Cancer Res.* 54 (1994) 908–914.
- [3] J.M. Connor, M.A. Ferguson-Smith, In: M.A. Ferguson-Smith (Ed.), *Essential Medical Genetics*, Blackwell Scientific Publications, Oxford, 1993, pp. 104–110.
- [4] T.D. Gelehrter, F.S. Collins, *Principles of Medical Genetics*, Williams and Wilkins, 1990.
- [5] C.R. Boland, L. Ricciardiello, *Proc. Natl. Acad. Sci. U.S.A.* 96 (1999) 14675–14677.
- [6] K.H. Kraemer, M.M. Lee, J. Scotto, *Arch. Dermatol.* 123 (1987) 241–250.
- [7] P. Jensen, S. Hansen, B. Moller, T. Leivestad, P. Pfeffer, O. Geiran, P. Fauchald, S. Simonsen, *J. Am. Acad. Dermatol.* 40 (1999) 177–186.
- [8] R.S. Schwartz, *J. Natl. Cancer Inst. Monogr.* 28 (2000) 5–9.
- [9] S.A. Birkeland, H.H. Storm, L.U. Lamm, L. Barlow, I. Blohme, B. Forsberg, B. Eklund, O. Fjeldborg, M. Friedberg, L. Frodin, et al., *Int. J. Cancer* 60 (1995) 183–189.
- [10] C.C. Harris, *Cancer Res.* 51 (1991) 5023s–5044s.
- [11] S.D. Hursting, T.J. Slaga, S.M. Fischer, J. DiGiovanni, J.M. Phang, *J. Natl. Cancer Inst.* 91 (1999) 215–225.
- [12] W.C. Hahn, R.A. Weinberg, *Nat. Rev. Cancer* 2 (2002) 331–341.
- [13] H. Jensen, J.L. Madsen, *Acta Med. Scand.* 223 (1988) 293–304.
- [14] M.R. Clemens, *Klin. Wochenschr.* 69 (1991) 1123–1134.
- [15] B.N. Ames, M. Profet, L.S. Gold, *Proc. Natl. Acad. Sci. U.S.A.* 87 (1990) 7777–7781.
- [16] L.S. Gold, T.H. Slone, B.R. Stern, N.B. Manley, B.N. Ames, *Science* 258 (1992) 261–265.
- [17] B.N. Ames, *Science* 221 (1983) 1256–1264.
- [18] B.N. Ames, L.S. Gold, *Science* 249 (1990) 970–971.
- [19] T.M. Florence, *Proc. Nutr. Aust.* 15 (1990) 88–93.
- [20] M. Polverelli, R. Teoule, *Z. Naturforsch. [C]* 29 (1974) 12–15.
- [21] B.I. Carr, *Cancer* 55 (1985) 218–224.
- [22] M.P. Rayman, *Lancet* 356 (2000) 233–241.
- [23] B.N. Ames, *Proc. Natl. Acad. Sci. U.S.A.* 96 (1999) 12216–12218.
- [24] T.J. Key, N.E. Allen, E.A. Spencer, R.C. Travis, *Lancet* 360 (2002) 861–868.
- [25] B.N. Ames, P. Wakimoto, *Nat. Rev. Cancer* 2 (2002) 694–704.
- [26] A.B. Awad, C.S. Fink, *J. Nutr.* 130 (2000) 2127–2130.
- [27] R. Nowak, *Science* 264 (1994) 500–501.
- [28] D. Schottenfeld, J.F. Fraumeni, *Cancer Epidemiology and Prevention*, second ed., Oxford University Press, New York, NY, 1996.
- [29] W.C.R. Fund, *American Institute for Cancer Research*, Washington, DC, 1997, pp. 136–142.
- [30] G.S. Omenn, G.E. Goodman, M.D. Thornquist, J. Balmes, M.R. Cullen, A. Glass, J.P. Keogh, F.L. Meyskens, B. Valanis, J.H. Williams, S. Barnhart, S. Hammar, *N. Engl. J. Med.* 334 (1996) 1150–1155.
- [31] O.P. Heinonen, J.K. Huttunen, D. Albanes, J. Haapakoski, J. Palmgren, P. Pietinen, J. Pikkarainen, M. Rautalahti, J. Virtamo, B.K. Edwards, P. Greenwald, A.M. Hartman, P.R. Taylor, J. Haukka, P. Jarvinen, N. Malila, S. Rapola, P. Jokinen, J. Karjalainen, J. Launonen, J. Mutikainen, M. Sarjakoski, A. Suorsa, M. Tiainen, M. Verkasalo, et al., *N. Engl. J. Med.* 330 (1994) 1029–1035.
- [32] P.A. Cerutti, *Science* 227 (1985) 375–381.
- [33] I. Fridovich, *Annu. Rev. Pharmacol. Toxicol.* 23 (1983) 239–257.
- [34] J. Stoehlmacher, D.J. Park, W. Zhang, S. Groshen, D.D. Tsao-Wei, M.C. Yu, H.J. Lenz, *J. Natl. Cancer Inst.* 94 (2002) 936–942.
- [35] B. Ketterer, *Mutat. Res.* 202 (1988) 343–361.
- [36] F. Oesch, J. Doehmer, T. Friedberg, H. Glatt, B. Oesch-Bartlomowicz, K.L. Platt, P. Steinberg, D. Utesch, H. Thomas, *Prog. Clin. Biol. Res.* 340B (1990) 49–65.
- [37] M.O. James, J.D. Schell, S.M. Boyle, A.H. Altman, E.A. Cromer, *Chem. Biol. Interact.* 79 (1991) 305–321.
- [38] F.P. Perera, *Science* 278 (1997) 1068–1073.
- [39] D.M. Gertig, D.J. Hunter, *Semin. Cancer Biol.* 8 (1998) 285–298.
- [40] F.J. Gonzalez, D.W. Nebert, *Trends Genet.* 6 (1990) 182–186.
- [41] P. Borst, R. Evers, M. Kool, J. Wijnholds, *J. Natl. Cancer Inst.* 92 (2000) 1295–1302.
- [42] J.R. Murren, *Clin. Cancer Res.* 8 (2002) 633–635.
- [43] B.I. Sikic, *J. Clin. Oncol.* 11 (1993) 1629–1635.
- [44] C.J. Link Jr., R.K. Burt, V.A. Bohr, *Cancer Cells* 3 (1991) 427–436.
- [45] R.D. Wood, *Annu. Rev. Biochem.* 65 (1996) 135–167.
- [46] J.H. Hoeijmakers, *Nature* 411 (2001) 366–374.
- [47] P.C. Hanawalt, A. Sarasin, *Trends Genet.* 2 (1986) 124–129.
- [48] J.H. Hoeijmakers, *Eur. J. Cancer* 30A (1994) 1912–1921.
- [49] A.R. Gennery, A.J. Cant, P.A. Jeggo, *Clin. Exp. Immunol.* 121 (2000) 1–7.
- [50] P. Schar, *Cell* 104 (2001) 329–332.
- [51] L.A. Loeb, K.R. Loeb, J.P. Anderson, *Proc. Natl. Acad. Sci. U.S.A.* 100 (2003) 776–781.
- [52] K. Munger, *Cancer Invest.* 20 (2002) 71–81.
- [53] D.A. Haber, E.R. Fearon, *Lancet* 351 (Suppl. 2) (1998) SIII–SIII8.
- [54] K. Macleod, *Curr. Opin. Genet. Dev.* 10 (2000) 81–93.
- [55] C.H. Streuli, *Breast Cancer Res.* 4 (2002) 137–140.
- [56] C.C. Harris, M. Hollstein, *N. Engl. J. Med.* 329 (1993) 1318–1327.
- [57] E.E. Balint, K.H. Vousden, *Br. J. Cancer* 85 (2001) 1813–1823.
- [58] O. Pluquet, P. Hainaut, *Cancer Lett.* 174 (2001) 1–15.
- [59] K.M. Ryan, A.C. Phillips, K.H. Vousden, *Curr. Opin. Cell Biol.* 13 (2001) 332–337.
- [60] W.S. el-Deiry, *Semin. Cancer Biol.* 8 (1998) 345–357.
- [61] K.H. Vousden, *Cell* 103 (2000) 691–694.
- [62] J. Marx, *Science* 261 (1993) 1385–1387.
- [63] X. Chen, L.J. Ko, L. Jayaraman, C. Prives, *Genes Dev.* 10 (1996) 2438–2451.
- [64] L.B. Owen-Schaub, W. Zhang, J.C. Cusack, L.S. Angelo, S.M. Santee, T. Fujiwara, J.A. Roth, A.B. Deisseroth, W.W. Zhang, E. Kruzel, et al., *Mol. Cell. Biol.* 15 (1995) 3032–3040.
- [65] G.S. Wu, T.F. Burns, E.R. McDonald III, W. Jiang, R. Meng, I.D. Krantz, G. Kao, D.D. Gan, J.Y. Zhou, R. Muschel, S.R. Hamilton, N.B. Spinner, S. Markowitz, G. Wu, W.S. el-Deiry, *Nat. Genet.* 17 (1997) 141–143.
- [66] K.F. Pirollo, K.B. Bouker, E.H. Chang, *Anticancer Drugs* 11 (2000) 419–432.
- [67] M. Hollstein, K. Rice, M.S. Greenblatt, T. Soussi, R. Fuchs, T. Sorlie, E. Hovig, B. Smith-Sorensen, R. Montesano, C.C. Harris, *Nucleic Acids Res.* 22 (1994) 3551–3555.
- [68] G. Lozano, S.J. Elledge, *Nature* 404 (2000) 24–25.
- [69] M.G. van Oijen, P.J. Slootweg, *Clin. Cancer Res.* 6 (2000) 2138–2145.
- [70] S.B. Baylin, J.G. Herman, *J. Natl. Cancer Inst.* 93 (2001) 664–665.

- [71] A. Sigal, V. Rotter, *Cancer Res.* 60 (2000) 6788–6793.
- [72] S. Bates, K.H. Vousden, *Cell. Mol. Life Sci.* 55 (1999) 28–37.
- [73] K. Shiraishi, S. Fukuda, T. Mori, K. Matsuda, T. Yamaguchi, C. Tanikawa, M. Ogawa, Y. Nakamura, H. Arakawa, *Cancer Res.* 60 (2000) 3722–3726.
- [74] M. Reiss, *Oncol. Res.* 9 (1997) 447–457.
- [75] G.C. Blobe, W.P. Schiemann, H.F. Lodish, *N. Engl. J. Med.* 342 (2000) 1350–1358.
- [76] M.P. de Caestecker, E. Piek, A.B. Roberts, *J. Natl. Cancer Inst.* 92 (2000) 1388–1402.
- [77] J.J. Letterio, A.B. Roberts, *Annu. Rev. Immunol.* 16 (1998) 137–161.
- [78] E.R. Fearon, *J. Natl. Cancer Inst.* 92 (2000) 515–517.
- [79] M. Esteller, J.M. Silva, G. Dominguez, F. Bonilla, X. Matias-Guiu, E. Lerma, E. Bussaglia, J. Prat, I.C. Harkes, E.A. Repasky, E. Gabrielson, M. Schutte, S.B. Baylin, J.G. Herman, *J. Natl. Cancer Inst.* 92 (2000) 564–569.
- [80] G. Tamura, J. Yin, S. Wang, A.S. Fleisher, T. Zou, J.M. Abraham, D. Kong, K.N. Smolinski, K.T. Wilson, S.P. James, S.G. Silverberg, S. Nishizuka, M. Terashima, T. Motoyama, S.J. Meltzer, *J. Natl. Cancer Inst.* 92 (2000) 569–573.
- [81] M. Tamura, J. Gu, H. Tran, K.M. Yamada, *J. Natl. Cancer Inst.* 91 (1999) 1820–1828.
- [82] D. Hanahan, R.A. Weinberg, *Cell* 100 (2000) 57–70.
- [83] C.H. Buys, *N. Engl. J. Med.* 342 (2000) 1282–1283.
- [84] J. Wang, L.Y. Xie, S. Allan, D. Beach, G.J. Hannon, *Genes Dev.* 12 (1998) 1769–1774.
- [85] E. Hiyama, K. Hiyama, *Oncogene* 21 (2002) 643–649.
- [86] J.D. Henson, A.A. Neumann, T.R. Yeager, R.R. Reddel, *Oncogene* 21 (2002) 598–610.
- [87] T.M. Bryan, A. Englezou, J. Gupta, S. Bacchetti, R.R. Reddel, *EMBO J.* 14 (1995) 4240–4248.
- [88] I.J. Fidler, L.M. Ellis, *Cell* 79 (1994) 185–188.
- [89] P. Carmeliet, R.K. Jain, *Nature* 407 (2000) 249–257.
- [90] K.J. O’Byrne, A.G. Dalglish, M.J. Browning, W.P. Steward, A.L. Harris, *Eur. J. Cancer* 36 (2000) 151–169.
- [91] J. Rak, Y. Mitsuhashi, C. Sheehan, A. Tamir, A. Vioria-Petit, J. Filmus, S.J. Mansour, N.G. Ahn, R.S. Kerbel, *Cancer Res.* 60 (2000) 490–498.
- [92] F. Okada, J.W. Rak, B.S. Croix, B. Lieubeau, M. Kaya, L. Roncari, S. Shirasawa, T. Sasazuki, R.S. Kerbel, *Proc. Natl. Acad. Sci. U.S.A.* 95 (1998) 3609–3614.
- [93] G. Breier, S. Blum, J. Peli, M. Groot, C. Wild, W. Risau, E. Reichmann, *Int. J. Cancer* 97 (2002) 142–148.
- [94] P.H. Maxwell, M.S. Wiesener, G.W. Chang, S.C. Clifford, E.C. Vaux, M.E. Cockman, C.C. Wykoff, C.W. Pugh, E.R. Maher, P.J. Ratcliffe, *Nature* 399 (1999) 271–275.
- [95] K.M. Dameron, O.V. Volpert, M.A. Tainsky, N. Bouck, *Science* 265 (1994) 1582–1584.
- [96] R. Ravi, B. Mookerjee, Z.M. Bhujwalla, C.H. Sutter, D. Artemov, Q. Zeng, L.E. Dillehay, A. Madan, G.L. Semenza, A. Bedi, *Genes Dev.* 14 (2000) 34–44.
- [97] G.L. Semenza, *Annu. Rev. Cell. Dev. Biol.* 15 (1999) 551–578.
- [98] P. Friedl, K. Wolf, *Nat. Rev. Cancer* 3 (2003) 362–374.
- [99] L.A. Liotta, P.S. Steeg, W.G. Stetler-Stevenson, *Cell* 64 (1991) 327–336.
- [100] A.F. Chambers, A.C. Groom, I.C. MacDonald, *Nat. Rev. Cancer* 2 (2002) 563–572.
- [101] L.A. Liotta, R.H. Goldfarb, R. Brundage, G.P. Siegal, V. Terranova, S. Garbisa, *Cancer Res.* 41 (1981) 4629–4636.
- [102] J.R. MacDougall, L.M. Matrisian, *Cancer Metastasis Rev.* 14 (1995) 351–362.
- [103] V.M. Kahari, U. Saarialho-Kere, *Ann. Med.* 31 (1999) 34–45.
- [104] D. Boyd, *Cancer Metastasis Rev.* 15 (1996) 77–89.
- [105] G. Ratzinger, P. Stoitner, S. Ebner, M.B. Lutz, G.T. Layton, C. Rainer, R.M. Senior, J.M. Shipley, P. Fritsch, G. Schuler, N. Romani, *J. Immunol.* 168 (2002) 4361–4371.
- [106] Z. Werb, *Cell* 91 (1997) 439–442.
- [107] K. Gohil, L. Rothfuss, J. Lang, L. Packer, *J. Appl. Physiol.* 63 (1987) 1638–1641.
- [108] R. Fodde, *Eur. J. Cancer* 38 (2002) 867–871.
- [109] A.M. Arias, *Cell* 105 (2001) 425–431.
- [110] K. Nakanishi, T. Kawai, C. Torikata, T. Aurues, T. Ikeda, *Cancer* 82 (1998) 724–732.
- [111] B.A. Yoshida, M.M. Sokoloff, D.R. Welch, C.W. Rinker-Schaeffer, *J. Natl. Cancer Inst.* 92 (2000) 1717–1730.
- [112] P.S. Steeg, G. Bevilacqua, L. Kopper, U.P. Thorgerisson, J.E. Talmadge, L.A. Liotta, M.E. Sobel, *J. Natl. Cancer Inst.* 80 (1988) 200–204.
- [113] S. Cheng, M.A. Alfonso-Jaume, P.R. Mertens, D.H. Lovett, *Biochem. J.* 366 (2002) 807–816.
- [114] J. Grossmann, *Apoptosis* 7 (2002) 247–260.
- [115] S.M. Frisch, R.A. Screaton, *Curr. Opin. Cell. Biol.* 13 (2001) 555–562.
- [116] Y. Feng, L.Y. Wang, T. Cai, J.W. Jin, G.F. Zhou, L.H. Cao, X.L. Zha, *J. Exp. Clin. Cancer Res.* 20 (2001) 429–438.
- [117] D.G. Stupack, X.S. Puente, S. Boutsaboualoy, C.M. Storgard, D.A. Cheresch, *J. Cell. Biol.* 155 (2001) 459–470.
- [118] G. Fiucci, D. Ravid, R. Reich, M. Liscovitch, *Oncogene* 21 (2002) 2365–2375.
- [119] Z. Weng, M. Xin, L. Pablo, D. Grueneberg, M. Hagel, G. Bain, T. Muller, J. Papkoff, *J. Biol. Chem.* 277 (2002) 18677–18686.
- [120] M.L. Coll, K. Rosen, V. Ladedá, J. Filmus, *Oncogene* 21 (2002) 2908–2913.
- [121] J. Laurence, *AIDS Read* 13 (2003) 202, 205.
- [122] C. Boshoff, R. Weiss, *Nat. Rev. Cancer* 2 (2002) 373–382.
- [123] J.M. Holland, T.J. Mitchell, L.C. Gipson, M.S. Whitaker, *J. Natl. Cancer Inst.* 61 (1978) 1357–1361.
- [124] S.A. Weitzman, A.B. Weitzberg, E.P. Clark, T.P. Stossel, *Science* 227 (1985) 1231–1233.
- [125] T. Tamatani, P. Turk, S. Weitzman, R. Oyasu, *Jpn. J. Cancer Res.* 90 (1999) 829–836.
- [126] F.A. Fitzpatrick, *Int. Immunopharmacol.* 1 (2001) 1651–1667.
- [127] E. Shacter, S.A. Weitzman, *Oncology (Huntingt)* 16 (2002) 217–226, 229, 230–212 (discussion).
- [128] T.A. Fehniger, K. Suzuki, A. Ponnappan, J.B. VanDeusen, M.A. Cooper, S.M. Florea, A.G. Freud, M.L. Robinson, J. Durbin, M.A. Caligiuri, *J. Exp. Med.* 193 (2001) 219–231.
- [129] E.Y. Lin, A.V. Nguyen, R.G. Russell, J.W. Pollard, *J. Exp. Med.* 193 (2001) 727–740.
- [130] M. Suganuma, S. Okabe, M.W. Marino, A. Sakai, E. Sueoka, H. Fujiki, *Cancer Res.* 59 (1999) 4516–4518.
- [131] R.J. Moore, D.M. Owens, G. Stamp, C. Arnott, F. Burke, N. East, H. Holdsworth, L. Turner, B. Rollins, M. Pasparakis, G. Kollias, F. Balkwill, *Nat. Med.* 5 (1999) 828–831.
- [132] D.M. Hilbert, M. Kopf, B.A. Mock, G. Kohler, S. Rudikoff, *J. Exp. Med.* 182 (1995) 243–248.
- [133] J.D. Hudson, M.A. Shoaibi, R. Maestro, A. Carnero, G.J. Hannon, D.H. Beach, *J. Exp. Med.* 190 (1999) 1375–1382.
- [134] J.J. Mule, J.K. McIntosh, D.M. Jablons, S.A. Rosenberg, *J. Exp. Med.* 171 (1990) 629–636.
- [135] K. Haranaka, N. Satomi, A. Sakurai, *Int. J. Cancer* 34 (1984) 263–267.
- [136] W. Lasek, A. Wankowicz, K. Kuc, W. Feleszko, J. Gołab, A. Giermasz, W. Wiktor-Jedrejczak, M. Jakóbsiak, *Cancer Immunol. Immunother.* 40 (1995) 315–321.
- [137] W. Lasek, J. Gołab, W. Maslinski, T. Switaj, E.Z. Balkowiec, T. Stokłosa, A. Giermasz, M. Malejczyk, M. Jakóbsiak, *Eur. Cytokine Netw.* 10 (1999) 345–356.
- [138] L.J. Marnett, R.N. DuBois, *Annu. Rev. Pharmacol. Toxicol.* 42 (2002) 55–80.
- [139] C.S. Williams, M. Mann, R.N. DuBois, *Oncogene* 18 (1999) 7908–7916.

- [140] S.M. Prescott, F.A. Fitzpatrick, *Biochim. Biophys. Acta* 1470 (2000) M69–78.
- [141] S. Ambis, S.P. Hussain, C.C. Harris, *FASEB J.* 11 (1997) 443–448.
- [142] D. Berd, M.J. Mastrangelo, E. Lattime, T. Sato, H.C. Maguire Jr., *Cancer Immunol. Immunother.* 42 (1996) 263–267.
- [143] L. Zhang, T. Yoshimura, D.T. Graves, *J. Immunol.* 158 (1997) 4855–4861.
- [144] F. Fioretti, D. Fradelizi, A. Stoppacciaro, S. Ramponi, L. Ruco, A. Minty, S. Sozzani, C. Garlanda, A. Vecchi, A. Mantovani, *J. Immunol.* 161 (1998) 342–346.
- [145] E. Di Carlo, G. Forni, P. Lollini, M.P. Colombo, A. Modesti, P. Musiani, *Blood* 97 (2001) 339–345.
- [146] D.I. Gabrilovich, M.P. Velders, E.M. Sotomayor, W.M. Kast, *J. Immunol.* 166 (2001) 5398–5406.
- [147] J. Gołab, R. Zagodzón, T. Stoklosa, A. Kaca, A. Dabrowska, A. Giermasz, W. Feleszko, M. Jakóbiśiak, *Neoplasma* 45 (1998) 35–39.
- [148] S.N. Bock, R.B. Cameron, P. Kragel, J.J. Mule, S.A. Rosenberg, *Cancer Res.* 51 (1991) 2649–2654.
- [149] D.F. Bajorin, N.K. Cheung, A.N. Houghton, *Semin. Hematol.* 28 (1991) 42–48.
- [150] S.K. Chambers, Y. Wang, R.E. Gertz, B.M. Kacinski, *Cancer Res.* 55 (1995) 1578–1585.
- [151] W. Feleszko, A. Giermasz, J. Gołab, W. Lasek, K. Kuc, M. Szperl, M. Jakóbiśiak, *Cancer Lett.* 101 (1996) 193–197.
- [152] A.S. Haqqani, J.K. Sandhu, H.C. Birnboim, *Neoplasia* 2 (2000) 561–568.
- [153] L.M. Coussens, C.L. Tinkle, D. Hanahan, Z. Werb, *Cell* 103 (2000) 481–490.
- [154] K. Iwasaki, M. Torisu, T. Fujimura, *Cancer* 58 (1986) 1321–1327.
- [155] R. Andreesen, B. Hennemann, S.W. Krause, *J. Leukoc. Biol.* 64 (1998) 419–426.
- [156] S.D. Lucas, A. Karlsson-Parra, B. Nilsson, L. Grimelius, G. Akerstrom, J. Rastad, C. Juhlin, *Hum. Pathol.* 27 (1996) 1329–1335.
- [157] F. Niculescu, H.G. Rus, M. Retegan, R. Vlaicu, *Am. J. Pathol.* 140 (1992) 1039–1043.
- [158] G.A. Niehans, D.L. Cherwitz, N.A. Staley, D.J. Knapp, A.P. Dalmaso, *Am. J. Pathol.* 149 (1996) 129–142.
- [159] M. Yamakawa, K. Yamada, T. Tsuge, H. Ohnui, T. Ogata, M. Dobashi, Y. Imai, *Cancer* 73 (1994) 2808–2817.
- [160] J.M. Leiden, B.A. Karpinski, L. Gottschalk, J. Kornbluth, *J. Immunol.* 142 (1989) 2140–2147.
- [161] M.I. Nishimura, I. Stroynowski, L. Hood, S. Ostrand-Rosenberg, *J. Immunol.* 141 (1988) 4403–4409.
- [162] J. Pena, C. Alonso, R. Solana, R. Serrano, J. Carracedo, R. Ramirez, *Eur. J. Immunol.* 20 (1990) 2445–2448.
- [163] J. Pena, R. Solana, M.C. Alonso, M. Santamaria, R. Serrano, R. Ramirez, *J. Immunogenet.* 16 (1989) 407–412.
- [164] D. Pende, S. Parolini, A. Pessino, S. Sivori, R. Augugliaro, L. Morelli, E. Marcenaro, L. Accame, A. Malaspina, R. Biassoni, C. Bottino, L. Moretta, A. Moretta, *J. Exp. Med.* 190 (1999) 1505–1516.
- [165] S.S. Farag, T.A. Fehniger, L. Ruggeri, A. Velardi, M.A. Caligiuri, *Blood* 100 (2002) 1935–1947.
- [166] F. Colucci, J.P. Di Santo, P.J. Leibson, *Nat. Immunol.* 3 (2002) 807–813.
- [167] A. Cerwenka, A.B. Bakker, T. McClanahan, J. Wagner, J. Wu, J.H. Phillips, L.L. Lanier, *Immunity* 12 (2000) 721–727.
- [168] S. Bauer, V. Groh, J. Wu, A. Steinle, J.H. Phillips, L.L. Lanier, T. Spies, *Science* 285 (1999) 727–729.
- [169] J. Wu, Y. Song, A.B. Bakker, S. Bauer, T. Spies, L.L. Lanier, J.H. Phillips, *Science* 285 (1999) 730–732.
- [170] V. Groh, R. Rhinehart, H. Secrist, S. Bauer, K.H. Grabstein, T. Spies, *Proc. Natl. Acad. Sci. U.S.A.* 96 (1999) 6879–6884.
- [171] A. Diefenbach, E.R. Jensen, A.M. Jamieson, D.H. Raulet, *Nature* 413 (2001) 165–171.
- [172] S. Kim, K. Iizuka, H.L. Aguila, I.L. Weissman, W.M. Yokoyama, *Proc. Natl. Acad. Sci. U.S.A.* 97 (2000) 2731–2736.
- [173] J.C. Roder, T. Halotis, M. Klein, S. Korec, J.R. Jett, J. Ortaldo, R.B. Heberman, P. Katz, A.S. Fauci, *Nature* 284 (1980) 553–555.
- [174] J.L. Sullivan, K.S. Byron, F.E. Brewster, D.T. Purtilo, *Science* 210 (1980) 543–545.
- [175] J. Brittenden, S.D. Heys, J. Ross, O. Eremin, *Cancer* 77 (1996) 1226–1243.
- [176] W. Lasek, M. Jakóbiśiak, T. Stoklosa, *Transfusion* 34 (1994) 359–360.
- [177] N.E. Dunlap, V.G. Lane, G.A. Cloud, A.B. Tilden, *Cancer* 66 (1990) 1499–1504.
- [178] K. Imai, S. Matsuyama, S. Miyake, K. Suga, K. Nakachi, *Lancet* 356 (2000) 1795–1799.
- [179] T. Nakajima, N. Mizushima, K. Kanai, *Jpn. J. Clin. Oncol.* 17 (1987) 327–332.
- [180] S.P. Schantz, B.H. Campbell, O.M. Guillaumondegui, *Am. J. Surg.* 152 (1986) 467–474.
- [181] S.P. Schantz, H.E. Savage, T. Racz, D.L. Taylor, P.G. Sacks, *Am. J. Surg.* 158 (1989) 361–366.
- [182] P.I. Tartter, B. Steinberg, D.M. Barron, G. Martinelli, *Arch. Surg.* 122 (1987) 1264–1268.
- [183] H.F. Pross, E. Lotzova, *Nat. Immunol.* 12 (1993) 279–292.
- [184] P. Hersey, A. Edwards, G.W. Milton, W.H. McCarthy, *Nat. Immun. Cell. Growth Regul.* 3 (1983) 87–94.
- [185] S.P. Schantz, N.G. Ordóñez, *Nat. Immun. Cell. Growth Regul.* 10 (1991) 278–288.
- [186] M.J. Smyth, N.Y. Crowe, Y. Hayakawa, K. Takeda, H. Yagita, D.I. Godfrey, *Curr. Opin. Immunol.* 14 (2002) 165–171.
- [187] M. Exley, J. Garcia, S.P. Balk, S. Porcelli, *J. Exp. Med.* 186 (1997) 109–120.
- [188] R.R. Brutkiewicz, V. Sriram, *Crit. Rev. Oncol. Hematol.* 41 (2002) 287–298.
- [189] D.I. Godfrey, K.J. Hammond, L.D. Poulton, M.J. Smyth, A.G. Baxter, *Immunol. Today* 21 (2000) 573–583.
- [190] M.J. Smyth, D.I. Godfrey, *Nat. Immunol.* 1 (2000) 459–460.
- [191] M.J. Smyth, K.Y. Thia, S.E. Street, E. Cretney, J.A. Trapani, M. Taniguchi, T. Kawano, S.B. Pelikan, N.Y. Crowe, D.I. Godfrey, *J. Exp. Med.* 191 (2000) 661–668.
- [192] N.Y. Crowe, M.J. Smyth, D.I. Godfrey, *J. Exp. Med.* 196 (2002) 119–127.
- [193] M. Terabe, S. Matsui, N. Noben-Trauth, H. Chen, C. Watson, D.D. Donaldson, D.P. Carbone, W.E. Paul, J.A. Berzofsky, *Nat. Immunol.* 1 (2000) 515–520.
- [194] A.M. Moodycliffe, D. Nghiem, G. Clydesdale, S.E. Ullrich, *Nat. Immunol.* 1 (2000) 521–525.
- [195] M.J. Smyth, K.Y. Thia, S.E. Street, D. MacGregor, D.I. Godfrey, J.A. Trapani, *J. Exp. Med.* 192 (2000) 755–760.
- [196] W.F. Davidson, T. Giese, T.N. Fredrickson, *J. Exp. Med.* 187 (1998) 1825–1838.
- [197] S.M. Lippman, D.R. Parkinson, L.M. Itri, R.S. Weber, S.P. Schantz, D.M. Ota, M.A. Schusterman, I.H. Krakoff, J.U. Gutterman, W.K. Hong, *J. Natl. Cancer Inst.* 84 (1992) 235–241.
- [198] D.M. Shin, B.S. Glisson, F.R. Khuri, J.L. Clifford, G. Clayman, S.E. Benner, A.A. Forastiere, L. Ginsberg, D. Liu, J.J. Lee, J. Myers, H. Goepfert, R. Lotan, W.K. Hong, S.M. Lippman, *J. Clin. Oncol.* 20 (2002) 364–370.
- [199] J.L. Clifford, E. Walch, X. Yang, X. Xu, D.S. Alberts, G.L. Clayman, A.K. El-Naggar, R. Lotan, S.M. Lippman, *Clin. Cancer Res.* 8 (2002) 2067–2072.
- [200] T. Blankenstein, Z. Qin, *Curr. Opin. Immunol.* 15 (2003) 148–154.
- [201] A.S. Dighe, E. Richards, L.J. Old, R.D. Schreiber, *Immunity* 1 (1994) 447–456.
- [202] I. Gresser, F. Belardelli, C. Maury, M.T. Maunoury, M.G. Tovey, *J. Exp. Med.* 158 (1983) 2095–2107.
- [203] D.H. Kaplan, V. Shankaran, A.S. Dighe, E. Stockert, M. Aguet, L.J. Old, R.D. Schreiber, *Proc. Natl. Acad. Sci. U.S.A.* 95 (1998) 7556–7561.
- [204] S.E. Street, E. Cretney, M.J. Smyth, *Blood* 97 (2001) 192–197.

- [205] V. Shankaran, H. Ikeda, A.T. Bruce, J.M. White, P.E. Swanson, L.J. Old, R.D. Schreiber, *Nature* 410 (2001) 1107–1111.
- [206] S.E. Street, J.A. Trapani, D. MacGregor, M.J. Smyth, *J. Exp. Med.* 196 (2002) 129–134.
- [207] M. Ferrantini, F. Belardelli, *Semin. Cancer Biol.* 10 (2000) 145–157.
- [208] E.T. Creagan, J.S. Kovach, C.G. Moertel, S. Frytak, L.K. Kvols, *Cancer* 62 (1988) 2467–2471.
- [209] B. Knight, G.C. Yeoh, K.L. Husk, T. Ly, L.J. Abraham, C. Yu, J.A. Rhim, N. Fausto, *J. Exp. Med.* 192 (2000) 1809–1818.
- [210] A. Komori, J. Yatsunami, M. Suganuma, S. Okabe, S. Abe, A. Sakai, K. Sasaki, H. Fujiki, *Cancer Res.* 53 (1993) 1982–1985.
- [211] F. Balkwill, *Cytokine Growth Factor Rev.* 13 (2002) 135–141.
- [212] S. Wu, C.M. Boyer, R.S. Whitaker, A. Berchuck, J.R. Wiener, J.B. Weinberg, R.C. Bast Jr., *Cancer Res.* 53 (1993) 1939–1944.
- [213] H. Torisu, M. Ono, H. Kiryu, M. Furue, Y. Ohmoto, J. Nakayama, Y. Nishioka, S. Sone, M. Kuwano, *Int. J. Cancer* 85 (2000) 182–188.
- [214] R.D. Leek, R. Landers, S.B. Fox, F. Ng, A.L. Harris, C.E. Lewis, *Br. J. Cancer* 77 (1998) 2246–2251.
- [215] S.T. Malik, M.S. Naylor, N. East, A. Oliff, F.R. Balkwill, *Eur. J. Cancer* 26 (1990) 1031–1034.
- [216] K. Warzocha, G. Salles, J. Bienvenu, Y. Bastion, C. Dumontet, N. Renard, E.M. Neidhardt-Berard, B. Coiffier, *J. Clin. Oncol.* 15 (1997) 499–508.
- [217] M.S. Shin, H.S. Kim, S.H. Lee, W.S. Park, S.Y. Kim, J.Y. Park, J.H. Lee, S.K. Lee, S.N. Lee, S.S. Jung, J.Y. Han, H. Kim, J.Y. Lee, N.J. Yoo, *Cancer Res.* 61 (2001) 4942–4946.
- [218] M.J. Fisher, A.K. Virmani, L. Wu, R. Aplenc, J.C. Harper, S.M. Powell, T.R. Rebbeck, D. Sidransky, A.F. Gazdar, W.S. El-Deiry, *Clin. Cancer Res.* 7 (2001) 1688–1697.
- [219] E. Cretney, K. Takeda, H. Yagita, M. Glaccum, J.J. Peschon, M.J. Smyth, *J. Immunol.* 168 (2002) 1356–1361.
- [220] K. Takeda, M.J. Smyth, E. Cretney, Y. Hayakawa, N. Kayagaki, H. Yagita, K. Okumura, *J. Exp. Med.* 195 (2002) 161–169.
- [221] L.M. Sedger, M. Glaccum, J.C.L. Schuh, S.T. Kanaly, E. Williamson, N. Kayagaki, T. Yun, P. Smolak, T. Le, R. Goodwin, B. Gliniak, *Eur. J. Immunol.* 32 (2002) 2246–2254.
- [222] A. Strebel, F. Bachmann, M. Wernli, P. Erb, *Int. J. Cancer* 100 (2002) 627–634.
- [223] J. Gołab, R. Zagózdzon, *Int. J. Mol. Med.* 3 (1999) 537–544.
- [224] K. Boggio, G. Nicoletti, E. Dicarlo, F. Cavallo, L. Landuzzi, C. Melani, M. Giovarelli, I. Rossi, P. Nanni, C. Degiovanni, P. Bouchard, S. Wolf, A. Modesti, P. Musiani, P.L. Lollini, M.P. Colombo, G. Forni, *J. Exp. Med.* 188 (1998) 589–596.
- [225] Y. Noguchi, A. Jungbluth, E.C. Richards, L.J. Old, *Proc. Natl. Acad. Sci. U.S.A.* 93 (1996) 11798–11801.
- [226] J.H. Coggin, A.L. Barsoum, J.W. Rohrer, *Immunol. Today* 19 (1998) 405–408.
- [227] R.T. Prehn, J.M. Main, *J. Natl. Cancer Inst.* 18 (1957) 769–778.
- [228] T. Boon, *Adv. Cancer Res.* 58 (1992) 177–210.
- [229] P.L. Ward, H.K. Koeppen, T. Hurteau, D.A. Rowley, H. Schreiber, *Cancer Res.* 50 (1990) 3851–3858.
- [230] N. Renkvist, C. Castelli, P.F. Robbins, G. Parmiani, *Cancer Immunol. Immunother.* 50 (2001) 3–15.
- [231] I.D. Davis, M. Jefford, P. Parente, J. Cebon, *J. Leukoc. Biol.* 73 (2003) 3–29.
- [232] R.J. Papac, *In Vivo* 12 (1998) 571–578.
- [233] J.W. Uhr, R.H. Scheuermann, N.E. Street, E.S. Vitetta, *Nat. Med.* 3 (1997) 505–509.
- [234] T.G. Karrison, D.J. Ferguson, P. Meier, *J. Natl. Cancer Inst.* 91 (1999) 80–85.
- [235] A.J. Bremers, G. Parmiani, *Crit. Rev. Oncol. Hematol.* 34 (2000) 1–25.
- [236] M.J. Smyth, K.Y. Thia, E. Cretney, J.M. Kelly, M.B. Snook, C.A. Forbes, A.A. Scalzo, *J. Immunol.* 162 (1999) 6658–6662.
- [237] M. Girardi, D.E. Oppenheim, C.R. Steele, J.M. Lewis, E. Glusac, R. Filler, P. Hobby, B. Sutton, R.E. Tigelaar, A.C. Hayday, *Science* 294 (2001) 605–609.
- [238] S. Chouaib, C. Asselin-Paturel, F. Mami-Chouaib, A. Caignard, J.Y. Blay, *Immunol. Today* 18 (1997) 493–497.
- [239] F.H. Igney, P.H. Krammer, *J. Leukoc. Biol.* 71 (2002) 907–920.
- [240] A.G. Baxter, P.D. Hodgkin, *Nat. Rev. Immunol.* 2 (2002) 439–446.
- [241] H. Abken, A. Hombach, C. Heuser, K. Kronfeld, B. Seliger, *Trends Immunol.* 23 (2002) 240–245.
- [242] F. Garrido, F. Ruiz-Cabello, T. Cabrera, J.J. Perez-Villar, M. Lopez-Botet, M. Duggan-Keen, P.L. Stern, *Immunol. Today* 18 (1997) 89–95.
- [243] B. Seliger, M.J. Maeurer, S. Ferrone, *Immunol. Today* 18 (1997) 292–299.
- [244] J. Finke, S. Ferrone, A. Frey, A. Mufson, A. Ochoa, *Immunol. Today* 20 (1999) 158–160.
- [245] X.F. Bai, J. Liu, O. Li, P. Zheng, Y. Liu, *J. Clin. Invest.* 111 (2003) 1487–1496.
- [246] M. Nakashima, K. Sonoda, T. Watanabe, *Nat. Med.* 5 (1999) 938–942.
- [247] J. O’Connell, M.W. Bennett, G.C. O’Sullivan, J.K. Collins, F. Shanahan, *Immunol. Today* 20 (1999) 46–52.
- [248] W. Roth, S. Isenmann, M. Nakamura, M. Platten, W. Wick, P. Kleihues, M. Bahr, H. Ohgaki, A. Ashkenazi, M. Weller, *Cancer Res.* 61 (2001) 2759–2765.
- [249] T.L. Whiteside, *Semin. Cancer Biol.* 12 (2002) 43–50.
- [250] A. Gorter, S. Meri, *Immunol. Today* 20 (1999) 576–582.
- [251] T.L. Whiteside, *Cancer Immunol. Immunother.* 48 (1999) 346–352.