

GENE *P53* MUTATIONS, PROTEIN P53, AND ANTI-P53 ANTIBODIES AS BIOMARKERS OF CANCER PROCESS

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Abstract. The finding that gene mutations and changes in their expression form the basis of cancer processes, has prompted molecular epidemiologists to use biomarkers for detecting damaged genes or proteins synthesized under their control in easily available cellular material or systemic liquids. Mutations in the suppressor gene *p53* are thought to be essential for cancer development. This gene is one of the most important regulators of transcription, cellular cycle, DNA repair and apoptosis detected till now. Inactivation of gene *p53* leads to uncontrolled cell divisions, and further to transformation of normal cells into the carcinous ones. Observations that mutations in gene *p53* appear under conditions of occupational and environmental exposures to chemical and physical carcinogens, such as vinyl chloride, radon, or aflatoxin B1, have proved to be of enormous importance for the occupational and environmental health. Changes in expression of gene *p53*, and also its mutations, cause variations of cellular protein p53 concentration. Higher cellular protein p53 levels are associated with increased protein transfer to the extracellular liquid and to blood. It has been observed that increased blood serum protein p53 concentrations may have a prognostic value in early diagnosis of lung cancer. The results of a number of studies confirm that accumulation of a mutated form of protein p53, and presumably also large quantities of wild forms of that protein in the cells, may be a factor that triggers the production of anti-p53 antibodies. Statistical analysis showed that anti-p53 antibodies can be regarded as a specific biomarker of cancer process. The prevalence of anti-p53 antibodies correlated with the degree of cancer malignancy. The increased incidence of anti-p53 antibodies was also associated with higher frequency of mutations in gene *p53*. There are some reports confirming that anti-p53 antibodies emerging in blood serum in the subclinical phase of cancer development may be associated with the occupational exposure to the carcinogenic agents.

Key words:

Molecular epidemiology, Gen *p53* mutations, Protein p53, Anti-p53 antibodies

INTRODUCTION

Detection of pre-clinical changes, which precede the development of the overt form of a disease and identification of measurable indicators signaling such changes in people exposed to occupational and environmental pollutants, constitutes one of the principal research aims of molecular epidemiology. The latter makes use of suitable biological indicators (biomarkers) as basic research tools

to monitor pathological processes during the latent stage of the disease in people exposed to harmful agents [1,2]. The finding that gene damage (mutations and changes in their expression) forms the basis of cancer processes, has prompted molecular epidemiologists to use biomarkers for detecting damaged genes (or proteins synthesized under their control) in easily available cellular material or systemic fluids. Currently available biomarkers of cancer process make it possible to detect exposure to carcino-

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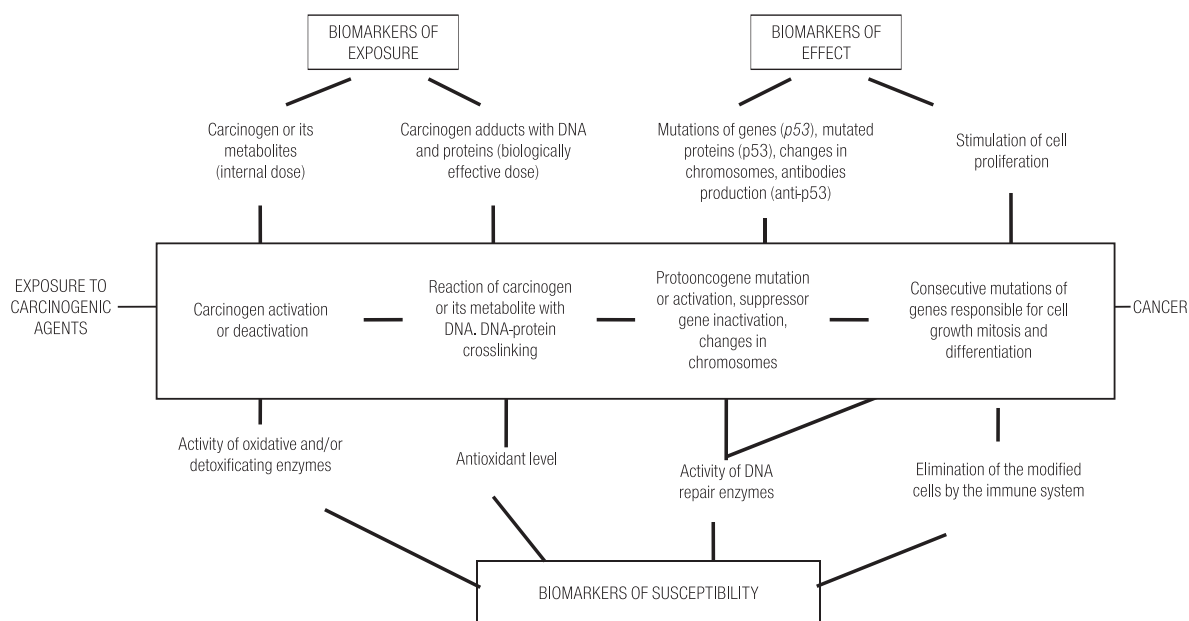


Fig. 1. Biomarkers of cancer process make it possible to detect exposure to carcinogens, to assess the extent of exposure, and to estimate carcinogen effects on cells and tissues of the exposed human organism.

gens, to assess the extent of the exposure, and to estimate carcinogen effects on cells and tissues of the exposed organism. It is also feasible to detect individual sensibility (genetically related or acquired) to the carcinogenic activity of various chemical and physical agents (Fig. 1) [3]. Currently available data on the molecular mechanisms by which normal cells become transformed into cancer ones show that accumulation of genetic defects in many genes, no matter whether they result from the hereditary processes, autonomous mutation, or else from the mutagenic and promotional effects of environmental agents, is a fundamental prerequisite for such transformation to occur. It seems highly probable that activation of protooncogenes, deactivation of suppressor genes, and the resultant qualitative and quantitative changes that occur in oncoproteins, which control cellular growth and division processes, form the principal mechanism by which environmental carcinogens initiate carcinogenesis.

MUTATIONS OF GENE *p53* AS A BIOMARKER OF CARCINOGENESIS

Mutations in the suppressor gene *p53* are thought to be essential for cancer development. Gene *p53* is the most

prominent tumor suppressor gene because it is mutated in about half of human cancer cases (Fig. 2). Gene *p53* is essential for controlling cell division, DNA replication and cell differentiation, and in scheduling cell necrosis (apoptosis). Gene *p53*, as negative regulator of growth, is often said to perform as “molecular gendarme” guarding the integrity of the cellular genome, able to block growth of cells with damaged DNA, thereby enabling activation of the repair mechanisms before new division cycle is started. When the repair mechanisms fail, gene *p53* activates the process of apoptosis to prevent the development of abnormal cells. Blocking of the normal function of gene *p53*, in addition to disturbances in transcription control mechanisms, may inhibit apoptosis and thus, may essentially contribute to cellular immortality. Currently available data on gene *p53* show that this gene may play a key role in the process of development of different human cancer types. Expression of gene *p53* takes place in every cell of the human body, and protein synthesized under its control plays an important role in regulation of cellular divisions; this protein is one of the most important factors in cellular life [4,5].

Gene *p53* has been isolated and its nucleotide sequence precisely determined. The locus of human gene *p53* is on

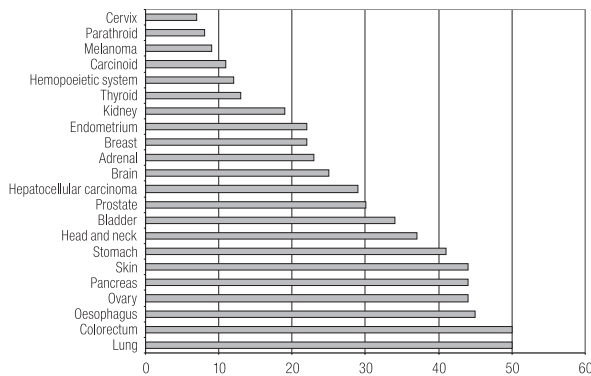


Fig. 2. Frequency of p53 mutations (%) in different human tumors.

chromosome 17p (17p13.1). This gene, comprising about 20 000 base pairs, is built of 11 exons, which code a 393 amino-acid-long protein product. Gene *p53* is one of the most important regulators of transcription, cellular cycle, DNA repair and apoptosis detected till now. Thus, the gene may be said to be the main factor inhibiting carcinogenesis in every tissue. Inactivation of gene *p53*, resulting in synthesis of inactive protein p53, leads to uncontrolled cell divisions, and further to transformation of normal cells into the carcinous ones. Very often such inactivation of gene *p53* results from mutation caused by exposure to environmental carcinogens. It should be noted that the gene *p53* inactivating mutations may sometimes be caused by endogenous factors (e.g. endogenously generated oxidants) and, in rare instances, these mutations may be hereditary. Mutations in the suppressor genes, including also gene *p53*, are recessive. Recessive mutation in gene *p53*, which leads to the synthesis of inactive proteins, is masked by active protein synthesized under control of the other ("wild") copy of that gene. Therefore, in order to enable the manifestation of the lost ability to inhibit hyperplasia, the mutation (or deletion) must also occur in the second wild copy. Mutations within gene *p53* are the most frequent (up to 50%) genetic defect found in cancers of all types. Distribution of the mutation within the gene is irregular. About 300 different point mutations have been found in gene *p53*, of which the majority (98%) are located in exons five to eight (codons 110 to 307). By comparing the frequency and location of the mutation in gene *p53*, five so called hot spots have been identified. They

comprise codons: 132 to 143, 151 to 159, 172 to 179, 237 to 249 and 272 to 286. Over 70% of all gene *p53* mutations have been observed to occur at those places [6].

It has been found that gene *p53* mutations induced by the endogenous agents differ from those caused by environmental agents. Thus, more than two third mutations found in gene *p53* isolated from cancer cells of colon are endogenous and characterized by the transition at places containing the cytosine-guanine (CpG) fragment. Over half of these transitions occur at three dinucleotide hot spots within codons 175, 248 and 273. Mutations in these three codons induce the replacement of arginine by some other amino acids. Mutations occurring at hot spots are not equally important for cancer development. Protein p53 synthesized under control of gene *p53* mutated in codon 175 shows three- to ten-fold higher activity for cancer transformation than protein synthesized under control of gene *p53* mutated in codon 273 [4,5].

The observation that mutations in gene *p53* appear under conditions of occupational and environmental exposures to chemical and physical carcinogens, such as vinyl chloride, radon and its decay products or aflatoxin B1, have proved to be of enormous importance for the occupational medicine and environmental health. It has been particularly interesting to note that location of point mutation in gene *p53* is specific for each carcinogen type. Mutations of gene *p53* detected in cells from pulmonary cancer tissues of the former uranium mine workers (exposure to radiation emitted by radon) were predominantly A:T→T:A, G:C→C:G, C:G→A:T transversions. This type of mutation is usually caused by exogenous mutagens. It is worth noting that the G:C→T:A transversion found in carcinous cigarette smokers was not detected in the investigated group of miners with lung cancer, despite that all examined miners with lung cancer smoked cigarettes. The pattern of mutation in gene *p53* caused by the alpha ionising radiation associated with exposure to argon differs from that detected in cigarette smokers not exposed to radon. Another pattern of gene *p53* mutation was shown to occur in people with liver angiosarcoma associated with occupational exposure to vinyl chloride. Those mutations

comprised G:C→A:T transition and A:T→T:A transversion [6–12].

Primary damages induced in gene *p53* by UV radiation are associated with formation of pyrimidine dimers, such as cyclobutane, and pyrimidone-(6-4)-pyrimidine photoproduct. Both the pyrimidine dimer and photoproduct 6–4 enhance mutation. It has been demonstrated that transitions of C:G→T:A and C:G, C:G→T:A, T:A, occurring in these fragments of gene *p53*, rich in pyrimidine bases, are characteristic for UV pattern of mutation occurring in gene *p53* (and also gene *H-ras*) [13,14].

A study on a group of people with hepatic angiosarcoma exposed to vinyl chloride has shown that mutation in gene *p53* may, by many months or even years, precede overt clinical symptoms of the hepatic cancer. Some researchers suggest that determination of mutation pattern (finger printing) in gene *p53* may be used as an effective biomarker of exposure, making it possible not only to confirm that exposure to a carcinogen has occurred, but also to identify the involved carcinogen. Detection of the mutation may also be used as a biomarker of early health effect to detect early phases of carcinogenesis at the level of the cellular genome [15–19].

PROTEIN P53 AS A BIOMARKER OF CANCER PROCESS

The 53 000 molecular mass phosphoprotein is the product of gene *p53* expression. Acidic amino acids prevail at the amino end of the protein, while its carboxylic end contains numerous basic amino acids. This region also comprises the nuclear localization signal (NLS) sequence responsible for protein transportation in the cellular nucleus. A tetrameric structure constitutes the functional form of protein *p53*, found only within the area of the nucleus. The mutated protein can form heterodimers with normal protein, thereby extending the half-life of the latter. Protein *p53* is able to combine with other cellular proteins participating in gene transcription processes, such as TATA-BOX binding protein (TBP), replication protein A (RPA), heat shock cognate protein (HSC70), and mouse double minute protein (MDM2), the latter being a physi-

ological inactivator of protein *p53*. Its ability to bind onco-gene virus protein products provides another major (second only to mutations) mechanism for inactivating the normal function of protein *p53*. Protein *p53* itself may also show transcription factor characteristics and join specific sequences of DNA by the hydrophobic domain, which comprises amino acids from 100 to 300 [16,20].

Normal protein *p53* affects cellular cycle by influencing transcription of a range of genes coding the regulator proteins. This occurs through activation of growth inhibiting genes or deactivation of growth stimulating genes. As a transcription factor, protein *p53* activates transcription of gene *p21*. This gene codes protein *p21* which, by joining cyclin-dependent kinases, inhibits their function and prevents the cell from entering into phase S and passing from phase G2 to M, thereby provides sufficient time for the enzymes repairing damages of DNA strand to repair possible defects. If the repair is ineffective, protein *p53* can shift the cell to the path of scheduled death (apoptosis). High level of protein *p53* causes that the equilibrium of *bcl* gene family expression products is shifted in favor of protein Bax by reducing protein Bcl-2 activity. Protein Bcl-2 is thought to constitute the factor of cell survival, while protein Bax is responsible for scheduled cell death. Both proteins form active dimers, and protein *p53*-controlled non-equilibrium of their concentrations shift the cell to the paths of death or survival [21].

Changes in expression of gene *p53*, and also in its mutations, cause variations of cellular protein *p53* concentration. Increased cellular protein *p53* concentration is the most frequent effect of mutations present in gene *p53* coding sequences. That is so, because the half-life of normal protein *p53* is up to 20 min, while the mutated protein can last in the cell for up to 12–20 h. Higher cellular protein levels are associated with the increased protein transfer to the extracellular fluid and to blood. Reports on the behavior of protein *p53* in blood serum show that the protein is found in 10–30% of cancer cases, and the proportion varies, depending on the cancer type. A report has also been published in which the authors claim that protein *p53* was not present in blood of people with cancer [22–24].

Fontanini et al. [24] have obtained very promising results regarding the possibility of using blood serum protein p53 determinations as a biomarker of carcinogenesis. The authors determined its concentrations in 50 patients with non-small cell lung carcinoma. They have proved that there is a significant correlation between concentration of protein p53 in tumor cells and the concentration of this protein in blood serum.

In 1996, Hemminki et al. [25] published their study on variations of protein p53 concentration in 111 patients with asbestosis, cancer of lungs and mesothelioma. The majority of the patients had been regularly examined since 1981. The authors have demonstrated that elevated blood serum protein p53 concentration (above 200 pg/ml) can be detected many years before cancer becomes clinically overt. Husgafvel-Pursiainen et al. [26] used the same group of patients to assess the relationship between mutation in gene *p53* and the accumulation of protein p53 in the cells of the pulmonary tissue and blood serum protein p53 concentration. Mutated gene *p53* (exons 5–9 were analyzed) was found to be present in the material containing cancer cells obtained from the 5 of 18 subjects (28%). A higher content of protein p53 in cancer cells was detected in the 7 of 20 subjects (35%). The presence of protein p53 (over cut-off concentration) in blood serum was ascertained in the 4 of 11 examined patients (36%). The presence of blood serum protein p53 correlated with its presence in the cancer cells of the pulmonary tissue. It has been observed that the presence of protein p53 in blood serum may have a prognostic value in early diagnosis of lung cancer or mesothelioma (positive predictive value was 0.67 and the negative one was 0.83).

In 1997, the detection of elevated concentrations of protein p53 in blood serum of patients with clinically overt form of lung cancer who had been occupationally exposed to salts of hexavalent chromium, and in people exposed to such salts who did not show clinically overt symptoms of cancer was reported [27]. The increased concentrations of protein p53 were detected also in people occupationally exposed to vinyl chloride. The results obtained by Schneider et al. [7] did not confirm that determinations of blood serum protein p53 concentrations could be used as

a biomarker for detecting lung cancer in people occupationally exposed to radon and its decay products in uranium mines.

Concentration of protein p53 in blood serum and its concentration and excretion with urine were studied in patients with urinary bladder cancer and in the group of people occupationally exposed to genotoxic and mutagenic dyes. In the group of people with urinary bladder cancer, protein p53 was found to be present in blood serum of 47.6% of patients, while for urine the respective value was 61.8%. The value of blood serum or urine protein p53 concentration did not depend on the degree of tumor development. In the group of people occupationally exposed to mutagenic and genotoxic dyes, protein p53 was detected in urine of 41.3% of patients, while in the control group of healthy people not occupationally exposed to carcinogenic agents, protein p53 was detected only in 22.2% of persons [28].

ANTI-P53 ANTIBODIES AS BIOMARKERS OF CANCER PROCESS

The immune response against their own cancer cells in people with developed cancer may point to the presence of tumor antigens characteristic of those cells. The results of a number of works confirm that accumulation of a mutated form of protein p53, and presumably also large quantities of wild form of this protein in the cells, may be a factor triggering the production of anti-p53 antibodies. A detailed immunochemical analysis shows that protein p53 comprises a range of dominants against which specific antibodies may be produced. Those antibodies can recognize both wild and mutated forms of protein p53. The antibodies produced under the control of the mutated protein are not directed against all protein molecules, but only against certain configurations of atoms on their surface, i.e. against specific epitopes. Protein p53 molecule actually acts as a carrier of antigen determinants, which make only a small portion of the molecule and in their isolated state are not themselves immunogenic. According to some authors, anti-p53 antibodies appear in people whose cancer cells have accumulated protein p53 (in its wild or

mutated form) [29–36]. Other authors do not confirm that observation. In their opinion, the detected anti-p53 antibodies constitute non-specific response of the immune system. They argue that this phenomenon results from the presence of polyreactive antibodies reacting with many protein epitopes. Anti-p53 antibodies detectable in people with cancer belong to subclass IgM and are characterized by comparatively weak bond to protein p53 antigen determinants. They may represent the response of the immune system to large quantities of cellular antigens released from decaying cancer cells [32,37,38].

Although the mechanism that leads to the presence of anti-p53 antibodies in the blood of carcinous patients has not been explained, research workers are still very much interested in the phenomenon. The prevalence of anti-p53 antibodies in blood serum of people with confirmed cancer is lower than the incidence of increased concentrations of protein p53 (mutated form of the protein) and ranges from several to over 20%. The presence of anti-p53 antibodies in blood serum was for the first time detected by Crawford et al. [29] in women with clinically confirmed breast cancer. Anti-p53 antibodies were detected in the 14 of 155 women (9%). The antibodies were not detected in the control group (0 of 164 women). Further research confirmed the presence of anti-p53 antibodies in blood serum, but again only in some people with clinically overt cancer. Survival of the lung cancer patients with high level of anti-p53 antibody was considerably shorter than that of the lung cancer patients in whom the antibodies were not detected. During the recent 20 years, over 80 reports on the prevalence of anti-p53 antibodies in 18 different cancer locations have been published.

Soussi [32] compared the available data from the literature on the incidence of anti-p53 antibodies in people with cancer (9489 cases), in healthy people and in patients suffering from diseases other than cancer (2404 persons) (Table 1). His statistical analysis of these data showed that anti-p53 antibodies can be regarded as a specific biomarker of cancer process. The incidence of anti-p53 antibodies correlated with the degree of cancer malignancy, especially if accompanied by metastasis to the lymphatic nodes. The increased incidence of anti p53 antibodies was

also associated with a higher frequency of mutations in gene *p53*.

The frequency of anti-p53 antibodies in blood serum of people with diagnosed cancer is lower than that of elevated concentrations of protein p53 (mutated or wild form) and ranges from several to over 40%. The explanation of the anti-p53 antibodies presence in blood is rather complicated and as yet it is not clear why in some cancer patients whose cancer cells contain mutated protein p53, anti-p53 antibodies are detectable whereas in other patients they are not. Several authors confirmed that the failure to detect antibodies in cancer patients with mutated gene *p53* was not due to insufficient sensitivity or specificity of the detection methods, but it resulted from the fact that anti-p53 antibodies were really absent. Several authors [21,26,30] showed that in spite of similar type of cancer, identical mutation within gene *p53* and accumulation of protein p53 in cancer cells, anti-p53 antibodies were detectable in some patients, while in others they were absent. In cancer patients whose cancer cells contained mutated gene *p53*, anti-p53 antibodies were not present also during the late stage of the disease. This may suggest that the manifestation of the humoral response was connected with certain biological specificity of those people. We may guess that in case of identical mutation within gene *p53*, the immune response (production of anti-p53 antibodies) was, e.g. dependent on specific, individual ability of presenting antigen-modified proteins p53 by class I and II MHC molecules [39–41].

Factors causing that anti-p53 antibodies are detected in cancer patients whose tumor cells do not contain mutated protein p53 may be numerous; it would be unreasonable to exclude certain technological or methodological shortcomings connected with the specificity of the applied analytical procedures used to detect anti-p53 antibodies. The heterogenous character of the tumor tissue, because of which the particular cancer tissue fragment collected to test the presence of the mutation in gene *p53* does not contain the mutation may be regarded as another contributing factor. The original tumor with cells without mutated gene *p53* may be accompanied by metastases containing cells with mutated gene *p53*. In a situation like

Table 1. p53-Ab frequency in various types of cancer: statistical evaluation (according to Soussi) [32]

Status	Frequency of p53-Abs (%)	P ^a
Healthy	1.45	
Esophageal cancer	31.00	<10 ⁻⁴
Oral cancer	29.00	<10 ⁻⁴
Bladder cancer	27.50	<10 ⁻⁴
Colon cancer	24.70	<10 ⁻⁴
Hepatocellular carcinoma	21.20	<10 ⁻⁴
Ovarian cancer	22.00	<10 ⁻⁴
Lung cancer	17.00	<10 ⁻⁴
Breast cancer	14.70	<10 ⁻⁴
Gastric cancer	14.40	<10 ⁻⁴
Pancreatic cancer	9.20	<10 ⁻⁴
Multiple myeloma	6.30	<10 ⁻⁴
Lymphoma	7.70	<10 ⁻⁴
Leukemia	3.30	0.005
Glioma	4.20	0.03
Prostate cancer	2.70	NS
Testicular cancer	0.00	NA
Melanoma	0.00	NA
Hepatoma	0.00	NA
Total cancers	16.90	<10 ⁻⁴

^a Correlation between cancer patients and healthy individuals were tested by the χ^2 test. The levels of significance were set at $p < 0.05$.

NS – not significant.

NA – not applicable.

that, although gene *p53* mutations were not detected in the cells of the original tumor, anti-p53 antibodies might be detectable in blood. It is also worth noting that, in addition to comparatively high specificity of the antibodies, the serologic analysis itself is a general test, the final result of which depends on many factors [32,39,42–45].

The data available currently show that the appearance and accumulation of mutated protein p53 are one of the principal reasons for launching the humoral response associated with starting of the production of anti-p53 antibodies. It is likely that accumulation (by various reasons) of wild, non-mutated forms of protein p53 also plays a role in this process. The antibodies formed under those circumstances can recognize not only the wild, but also the mutated form of protein p53. Using a set of synthetic peptides corresponding with different fragments of human protein p53 chain polypeptide, epitopes of those proteins

recognized by anti-p53 antibodies were precisely mapped. A detailed analysis of several hundred samples of blood serum containing anti-p53 antibodies showed that they recognize mainly the epitopes located in the N-terminal end of human proteins p53 and, to a lesser extent, the epitopes in the carboxylic end. Only a small number of the antibodies were able to recognize the central portion of protein p53, although this region comprises the majority of the mutations. So called “hot spots” also appear in that region. These observations confirm the data obtained on mice, which produced monoclonal antibodies against epitopes located in the amino and carboxylic ends of human protein p53. Monoclonal antibodies specific to the central region of protein p53 were obtained only after the mice had been subjected to carefully controlled, selective immunization [31,32,46,47].

The following facts may be quoted to support the conjecture that accumulation of protein p53 is the main source of the humoral response to the protein present in people with cancer:

- the presence of immunodominant epitopes outside the “hot spots” for the occurrences of the mutation;
- the correlation between the quantity of protein p53 accumulated in cancer cells and the humoral response manifested by formation of anti-p53 antibodies;
- the similar humoral response in patients with cancer, irrespective of cancer type; and
- the similar profile of protein p53 antigen loci detected in people with cancer and in hyperimmunized animals.

Accumulation of protein p53 triggers the self-immunization process manifested by the occurrence of anti-p53 antibodies. In normal circumstances, the protein p53 level is very low, suggesting that the tolerance to endogenous proteins (if any) is very limited [48]. It is worth noting that protein p53 is a very strong antigen. To immunize mice by protein p53, small protein quantities are sufficient to obtain high level of antibodies. Recently it has been published that human cells contain proteins which are homologous to protein p53. Although the resemblance of the sequence refers only to certain fragments of the polypeptide chain, it would not be reasonable to exclude that anti-p53 antibodies can cross-react with those proteins or can



stimulate production of antibodies reacting with protein p53 [49,50].

Findings by Trivers et al. [38] seem to be interesting from the point of view of cancer risk in workers exposed to occupational carcinogens. The workers in this study hold a variety of positions providing high exposure to vinyl chloride for 12–34 years, including an extended period before 1974 (0–34 years) without protection from exposure, and a shorter period after 1974 (0–18 years) with protection.

In all, 148 serum samples were obtained from 92 workers (15 of them had angiosarcoma of the liver (ASL)). Fourteen serum samples (from nine individuals) were positive for the presence of serum anti-p53 antibodies. The five of 15 individuals with ASL were positive for anti-p53 antibodies. The four of 77 vinyl chloride exposed workers without diagnosed ASL were positive for anti-p53 antibodies. According to Trivers et al. [38] serum anti-p53 antibodies can predate clinical diagnosis of certain tumors, such as ASL, and may be useful in identifying individuals at high cancer risk, e.g. workers with occupational exposure to vinyl chloride. Although several authors report that it is feasible to use anti-p53 antibody determinations as a biomarker for detecting subclinical forms of cancer, e.g. in cigarette smokers [51], the report by Trivers et al. [38] is till now the only work confirming that anti-p53 antibodies appearing in blood serum in the subclinical phase of cancer development may be associated with the occupational exposure to carcinogenic agents. The crucial differences between normal and cancer cells stem from the discrete changes in specific genes controlling proliferation and tissue homeostasis. Over 100 such cancer-related genes have been discovered, several of which are implicated in the natural history of human cancer because they are consistently found mutated in tumor. The p53 tumor suppressor gene is the most striking example as it is mutated in about half of almost all cancer types arising from a wide spectrum of tissues. Over past 15 years, a tremendous amount of work has been performed on p53 gene. Such an effort has never been made for any other single gene.

One of the challenges of the next millennium is an early detection of tumors using highly sensitive assays with gene probes specific for tumor genetic alteration. Such approaches are still under development and remain costly. We believe that there is still space for serological assays such as blood serum protein p53 and anti-p53 antibodies. Use of such low-cost biomarkers may be important in cancer prevention. One of their disadvantages is the lack of sensitivity inasmuch as only 20–40% of patients with gene p53 mutations are positive to the presence of protein p53 and anti-p53 antibodies in blood serum. In this place we would like to quote Soussi [32] who says: “if we estimate that there are 8 million patients with various types of cancer throughout the world, and 50% of them have a mutation in their p53 gene, then we can deduce that about 1 million of these patients have p53-Abs, and protein p53 in blood serum”.

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