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REVIEW PAPERS

ONCOPROTEINS AS BIOMARKERS OF A PRECLINICAL FORM OF CANCER OF THE RESPIRATORY TRACT INDUCED BY ENVIRONMENTAL CARCINOGENS

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Abstract. Experimental data and clinical observations indicate that an increased expression of oncogenes or their point mutations play an essential role in the process of carcinogenesis. It was important to find out that environmental and occupational carcinogens activate cellular oncogenes and contribute to increased amounts or occurrence of mutated oncoproteins. The latter are responsible for activating mechanisms which further the neoplastic transformation of cells. The researches are mainly concerned about two oncoproteins: oncoprotein coded by the ras oncogene – called p21 protein and oncoprotein coded by the erbB-2 oncogen – called p185 protein.

Investigations performed on neoplastic cells show that the neoplastic transformation process involves not only the afore-said oncogenes and their oncoproteins but also other oncogenes, and that the process itself required activating of more than one oncogen.

At present, it is possible to use measurements of oncoproteins in the biological material which is easily available. Due to this fact, a number of works in which measurements of oncoproteins in blood serum were used to assess cancer risk in persons exposed to carcinogens present at the work place, have been published.

According to the mortality statistics, neoplasm of respiratory and intrathoracic organs is the most common cause of death in males. The number of deaths caused by this type of neoplasm exceeds the total number of deaths caused by neoplasm of colon, prostate, pancreas or stomach, the most frequent causes of death from cancer among men. In females, neoplasm of respiratory and intrathoracic organs takes the third place, after neoplasm of breast and colon, among causes of death. About 90% of respiratory neoplasm is generated by epithelial cells lining respiratory pathways. Most likely it results from their high proliferative activity and from

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their direct contact with harmful chemical factors which contaminate the environment. The latter aspect is of particular interest from the point of view of occupational and environmental medicine, bearing in mind that carcinogenic substances present at the work and habitation places evoke the neoplastic transformation of cells in the majority of respiratory neoplasms (15).

The neoplastic transformation of normal epithelial cells of the respiratory pathways can be induced by various chemical and physical agents. Nowadays, it is presumed that changes associated with the transformation of normal cells into neoplastic ones are genetically determined and that they result from structural alterations in the cellular genome (21). A certain sequence of steps must occur if an environmental carcinogen is to evoke the neoplastic transformation of cells. These include contact itself with a carcinogenic agent, its absorption by the organism, transport to the target cell, metabolism of the agent to a reactive metabolite (however, not all carcinogenic substances must be activated), and interaction with critical molecules (deoxyribonucleic acid – DNA) in the target cell. The interaction of carcinogen with DNA leads to some irreversible alterations in the cellular genome which may cause the production of chromosome breaks and rearrangements or the introduction of point mutations (22). So far, it has not been possible to define exactly what changes, at the level of the cellular genome together with epigenetic processes involving a multi-stage process of promotion and progression, influence the expression of the malignant phenotype. However, it is known that alterations in the cellular genome are not random but they apply to some genes or subsets of genes that contribute to the process of cellular oncogenesis (23). These genes can be then differentiated into those which stimulate growth and division of cells and those which, exert an inhibitory influence. Protooncogenes belong to the first group and antioncogenes to the other (18, 31).

The knowledge of the influence of environmental carcinogens on the cellular genome is, in fact, limited only to the process of protooncogenes activation, and little is known about their influence on the inactivation of antioncogenes. Nevertheless, the data collected thus far, help to understand the mechanism that may lead to the neoplastic transformation of cells initiated by environmental carcinogens. It seems likely that activation of some protooncogenes and related qualitative and quantitative changes in oncoproteins, involved in the control of growth and division of cells, is a basic mechanism which is used by environmental carcinogenes to evoke this transformation process (1, 25, 33).

Biochemical cellular systems involved in the regulation of growth and division of epithelial cells lining the respiratory pathways are linked in any ways with protooncogenes. Protooncogenes called also cellular oncogenes (cellular – "onc", abbr: c-"onc") are homologous with oncogenes acute transforming viruses (viral – "onc", abbr: v-"onc") and induce neoplasm in short time. There are many observations that prove a critical role of protooncogenes in the cellular physiology. One of the most important ones is that in different periods of growth and development of normal tissues an increased expression of protooncogenes is noted, and this process plays a crucial role in proliferation and differentiation of cells (4).

Protooncogenes encoded proteins, the so called oncoproteins, which effect various cell functions. The oncoproteins can be divided into five groups depending on their functions in a cell. The first group comprises proteins with polypeptyde

properties of growth factors. These proteins, secreted by cells, can stimulate the growth of other cells through interaction with specific receptors. The platelet-derived growth factor (PDGF) encoded by the *c-sis* protooncogene can be included, among others, into this group. The second group contains oncoproteins with properties of membrane receptors for growth factors. The epidermal growth factor (EGF) receptor could be identified in this group. The protein component of this receptor, responsible for EGF binding, is encoded by the c-erbB protooncogen. The third group is composed of signal transducing proteins of GTPase property; p21 proteins encoded by the *ras* oncogenes may serve as an example of this group oncoproteins. Protein kinases which participate in signal transduction via the phosphorylation of other intracellular proteins and which control important biological processes of a cell belong to the fourth group of oncoproteins. Protein kinases encoded by such protooncogenes as *src*, *fes* or *mos* can be identified in this group. The fifth group are nuclear proteins which bind to DNA and influence the expression of other genes, activating the process of DNA synthesis and stimulating the division of cells. In this group proteins encoded by such protooncogenes as *myc*, *myb*, *fos* and *jun* can be found (3, 5).

As appears from the classification presented, oncoproteins play an important role in transferring of the growth signal and any disturbance in their effective functioning can lead to the neoplastic transformation of cells.

The *ras* and *erb* B protooncogenes are also of particular interest to researchers involved in investigations of the effect of environmental carcinogenes on the cellular genome. This stems from the assumption that changes in their structure and in their protein products are the main cause of the neoplastic transformation of cells of the respiratory system induced by chemical carcinogenes.

The Ha – *ras* protooncogen was first identified in a man in short arms of chromosome 11 and the Ki – *ras* in short arms of chromosome 12. The *ras* proteins (appropriately encoded protooncogenes) of molecular weight 21 kd, hence called p21, are involved in the intracellular information system transferring mitogenic signals. The p21 protein *ras* which contains GTPase activity is similar in the structure and function to proteins G involved in the activition process of phospholipase C (2).

The *erb* B protooncogene controls the synthesis of membrane protein being a component of the receptor for EGF. There are three domains in this receptor: the first one – an extracellular domain which binds to EGF, the second – a transmembrane domain and the third one – an intracellular domain. The latter one shows the tyrosine kinase activity and it is involved in the transduction of a mitogenic signal (the second transmitter) in condition when the extracellular domain is occupied by EGF (12).

The data collected indicate that in the process of the neoplastic transformation of cells, the oncoproteins listed above, produce their effect through the action of their protein products. That can be done either by the increased expression of protooncogene, leading to an enhanced contents of an appropriate oncoprotein, or by the normal expression of a mutated protooncogene. The increased expression of protooncogenes may be caused by the amplification of the number of copies of the gene present in the cell or by the introduction into the cell of a gene deregulatory sequence which could cause uncontrolled and increased expression. A mutated protooncogene (oncogene) undergoes a normal controlled expression but its protein

product with changed properties produces disturbances in the appropriate functioning of mechanisms which control growth and division of cells (11, 26, 27).

Studies performed on cell cultures confirmed that oncogene protein products are responsible for the neoplastic transformation. Microinjections of p21 proteins into normal cells containing inactivated *ras* oncogene produced transient cell transformation. The cells gradually returned to phenotypic normality as p21 protein was degraded intracellulary. Alternately, when monoclonal antibody directed against activated p21 was introduced through microinjections into cells transformed by the *ras* oncogene, the cells temporarily returned to a normal phonotype. But the degradation of the proteolytic complex: antibody – mutated p21 protein, caused the recurrence of neoplastic cells (19).

The data available at present prove that the so called activation of a single protooncogene by both the increased expression and mutation is not sufficient for a complete neoplastic transformation. The changes obtained usually depend on producing a phenotype of "immortal" cells. Activation of two protooncogenes considerably increases the frequency of transformation (31, 33).

When comparing clones of the *ras* oncogene of neoplastic epithelial cells of the respiratory pathways and a corresponding protooncogene from cells of normal epithelium, it was found that the change in expression of the gene and the increased contents of the protein specific for the oncogene are not always necessary for the neoplastic process. It is likely that the changes in the first line structure (sequence of nucleotide) of protooncogene and its transformation into an active oncogene decide whether the cell is already neoplastic or not . That in turn, is linked with the change in the first line structure (sequence of amino-acids) of the protein product encoded by this gene. New protein of changed properties emerges in cancerous epithelial cells of the respiratory pathways (20, 23).

The restrictive analysis of the genome of normal and cancerous epithelial cells indicated that the element responsible for the effect of the neoplastic transformation is embodied in the c-Ha-*ras* oncogene 1, in the Xmal/Kpnl element 350 nucleotides long, corresponding to the first exon of this encogene. A comparative analysis of the nucleotide sequence of this element with its normal homologue detected the transversion of guanine (G) and thymine (T) in the 60th nucleotide. In the consequence, the triplet (12th codon) guanine-guanine-cytosine (GGC) encoding glycine is transformed into the triplet guanine-thymine-cytosine (GTC) encoding valine. Further studies revealed that the "activation" of the *ras* protooncogene is also linked with other point mutations, among others, in the 61st codon (25).

As mentioned earlier, the protein synthetised under the control of the *ras* oncogene and designated p21 protein contains GTPase activity. It binds GTP and causes its hydrolysis. The other activity related to the GTP hydrolysis hinders the first one. Because of that, the intracellular signal (initiated by the growth factor after its binding to the cellular receptor) transduced by p21 protein is sometimes discontinued. The p21 protein encoded by the c-Ha-*ras* protooncogen 1 is likely to function as Gproteins. They become an indirect link which transduce the signal from the molecule of epithelial receptor to the efector enzyme – adenyl cyclase. Binding of G protein to GTP exerts its activation and stimulates adenyl cyclase. If GTP becomes hydrolysed by p21 protein to GDP, G proteins return to a inactiv-

ated form (29). Some of researchers postulate, however, that the phospholipase C – an enzyme activating the degradation of phosphatidylinositol – and not adenyl cyclase is the efector enzyme for p21 protein (30). In their opinion, p21 protein is a crucial regulator of the metabolism in this group of compounds which play an extremely essential role in transducing an intracellular mitogenic signal from the receptor activated by growth factors to the cellular nuclei (Fig. 1).

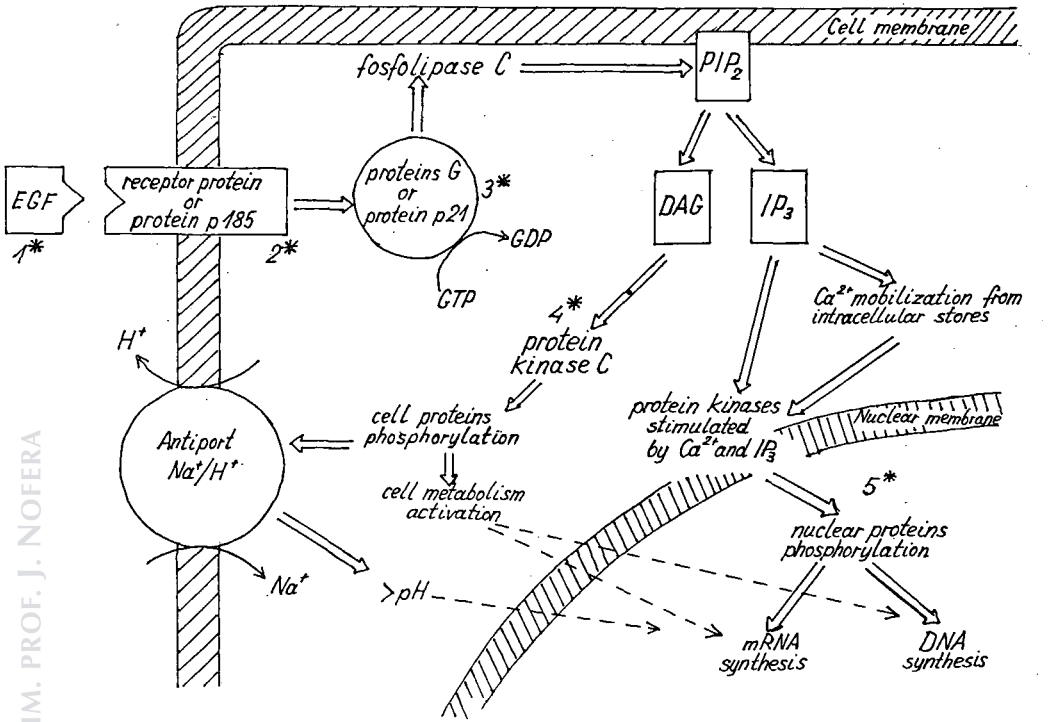

Fig. 1. The scheme of intracellular transduction of mitogenic signal induced by epithelial growth factor (EGF) in epithelial cells of the respiratory pathways. Stars mark the sites where mutated oncoproteins or their high concentrations can induce the transduction of mitogenic signal regardless of EGF.
PIP2 – phosphatidylinositol-4.5-diphosphoran;
IP3 – 1, 4, 5 triphosphoinositol; DAG – diacyloglycerol.

The p21 protein a product of mutated c-Ha-ras gene can also bind and hydrolyse GTP. But the normal protein contains GTPase activity that is, at least, one order of magnitude greater than that for the activated protein. The fact that it is not able to hydrolyse GTP efficiently is the reason why the mitotic signal, in the presence of the changed p21 protein in the cell, finds itself in some kind of a trap. The endpoint protein in which adenyl cyclase or phospholipase C can be found, is constantly stimulated by activated p21 protein. Therefore, a high level of cyclic AMP or a high rate of phosphatidylinositol degradation was retained. In consequence, the activity of protein kinase which plays a significant role in the process of cell division is stimulated (17, 24).

It should be noted that the presence of the changed *ras* p21 protein was found only in some cancerous epithelial cells isolated from the respiratory pathways. That indicates the possibility of other changes in the genome of cells present in the respiratory pathways which could be responsible for the neoplastic transformation. From studies carried out so far, it is clear that oncogene designated as c-erbB-2 which expresses its transformation activity through p185 protein is also significantly involved in the neoplastic transformation. This protein shows high homology of amino-acid sequence with protein producing a receptor for the epidermal growth factor (EGF). Like in the case of *ras* protooncogenes, activation of c-erbB-2 protooncogene and its transformation into oncogene can have the character of point mutation. This mutation causes that protein, which differs from normal protein by only one amino-acid at position 664 of the polypeptide chain, appears in the cell. This change in the amino-acid sequence is the reason why the EGF link is not necessary for transducing the signal of activation of the intracellular receptor. Under such conditions the cell becomes independent from extracellular EGF and a mitogenic signal activating the cellular proliferation is transduced from membrane receptors to EGF on a constant basis (24, 37, 32).

The correlation between activation of *ras* and erbB-2 protooncogenes and point mutations of those genes induced by well known chemical carcinogenes such as polycyclic aromatic hydrocarbons (PAH), nitrosoamines or aromatic amines proved to be an extremely important observation from the point of view of occupational medicine and environmental health (6, 7, 10). The division of cell uncontrolled by external factors seems to be one of the most significant consequences of changes in the cell genome induced by those carcinogenes.

A new concept of monitoring activated protooncogenes and related occurrence of increased amounts or changed oncoproteins in cells, in easily available biological fluids such as blood and urine, is of great value for environmental and occupational carcinogenesis. It provided new diagnostic methods in the assessment of carcinogenic effects of the occupational environment and human habitation places. Knowledge of the fact that oncoproteins can appear in biological fluids long before (many months or even years) clinical symptoms of lung tumors occur, provides new opportunities for taking more effective preventive measures (8, 16, 28).

Observations made during experiments in cell cultures which clearly indicate that increased amount of oncoproteins in cells is associated with their enhanced transfer to the extracellular fluid became a basis for measuring of oncoproteins in biological fluids (15). Similarly, the presence of changed oncoproteins in cells is reflected in the extracellular fluid which also contains small amounts of the protein (16).

Initial observations made in persons with clinical symptoms of lung cancer revealed the presence of increased amounts of oncoproteins or their mutated forms in plasma. It was also observed that increased amounts of oncoproteins retained in plasma after surgical treatment of lung cancer indicate the recurrence of malignant disease. It was confirmed as well that in a considerable proportion of persons without clinical symptoms of neoplasm but with oncoproteins present in plasma or excreted with urine the neoplastic disease was detected several months or even years later (8, 13).

The measurements of oncoproteins in plasma or urine in order to assess the risk of malignant disease of the respiratory pathways under conditions of occupational or environmental exposure to chemical carcinogenes have some limitations

associated with high cost of one measurement which requires highly specific monoclonal antibodies. Nevertheless, a number of works have been already published in which the authors try to prove the usefulness of those measurements in the prevention of respiratory neoplasms.

The measurement of oncoproteins in saliva – material very easily available – has been carried out i.a. in persons occupationally exposed to asbestos and in smokers, in other words in groups of high risk of lung cancer. The level of *ras* oncoprotein p21 was measured in saliva cells. In some persons p21 test was positive, however, they did not show any clinical symptoms of the respiratory neoplasm (8).

Very interesting studies on the measurement of oncoproteins in blood serum as a biomarker of early neoplastic changes were carried out by Brandt-Rauf et al. in 1990 (14). The authors examined 18 workers employed in a foundry and exposed to polycyclic aromatic hydrocarbons (PAH) (including benzo(a) pyrene) – well known occupational carcinogenes, in view of the presence of oncoproteins in blood serum. A high level of PAH adducts from DNA had been earlier found in the peripheral blood lymphocytes of those persons. This confirmed that those workers belonged to the group of a particularly high risk of malignant disease. The studies showed, among others, the presence of *fes* oncoproteins in the blood serum of a worker employed in the system of an eight-hour shift under condition of exposure to benzo(a) pyrene in concentration in ambient air exceeding 0.2 $\mu g/m^3$. In the serum of another worker also employed in a foundry and exposed to slightly higher concentrations of benzo(a)pyrene (0.05–0.2 $\mu g/m^3$) *fes* and *ras* oncoproteins were detected. The level of adducts in lymphocytes of these two workers was doubled in comparison with workers whose blood serum was free from oncoproteins. It should be stressed that according to data presented by the authors none of those workers showed clinical symptoms of neoplasm of the respiratory pathways or other organs.

Another study was performed in persons working in fire brigades and involved in anti-fire campaigns in chemical plants. Out of 33 persons covered by the study, oncoproteins, corresponding in their properties with β-transforming growth factor, were found in blood serum of 14 workers. It is worth mentioning that in none of controls non-occupationally exposed to chemical carcinogenes the presence of these oncoproteins were detected (8).

A similarly high per cent of persons with oncoproteins present in their blood serum was found in those exposed to polychlorinated biphenyles (PCBs) (9). This group was consisted of 16 workers involved in purification of transformer oil. The presence of *fes* oncoproteins was detected in six persons. It should be emphasized that all of them were smokers. In one of them *ras* oncoproteins and in another *sis* oncoproetins were found. Moreover, in two workers free from *fes* oncoproteins, the presence of *ras* oncoproteins was detected. The highest blood level of PCBs was found in the worker with *fes* and *ras* oncoproteins detected in his blood serum.

Data presented by Brandt-Rauf (8) on the significance of oncoprotein expression in the prognosis of malignant disease proved to be interesting. Studies were performed on samples of blood serum collected from 46 workers exposed to asbestos and silica. The samples were kept in the blood bank. Fourteen workers developed cancer from the time of blood collection (including 9 cases of the respiratory neoplasm). Usually, there was a period of 14 months between the blood collection

and its preservation in the blood bank, and the development of cancer. In the group of nine persons with lung cancer in as many as seven the presence of, at least, one oncoprotein was detected in blood serum collected earlier. One can draw a conclusion that the detection of increased expression of oncogene or its mutation through the measurement of oncoproteins in blood serum can be a useful biomarker in assessing the risk of cancer of the respiratory pathways (or other organs and tissues) in populations exposed to occupational and environmental carcinogens.

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