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Significance of genetic and immunologic studies in diagnosis and prevention of lung cancer

Znaczenie badań genetycznych i immunologicznych w profilaktyce i diagnostyce raka płuc

INTRODUCTION

The lung cancer is the most frequent neoplasm among men in many countries, and among women it also reaches a dominant position in certain countries. The lung cancer mortality rate in Polish men in the period 1983–87 was 64.7, and in women it was 8.2. One million of newly diagnosed lung cancer patients are identified every year in the world and more than 25 thousand in Poland. The epidemiological data confirm the association between tobacco smoking and the lung cancer epidemic, but the other risk factors for lung cancer are still vague [36].

There are no precise data about familial predisposition to lung cancer, but several cancer familial syndromes (CFS) that include lung cancer have been identified. The lung cancer appear more frequently in families, which members suffer from breast, stomach, larynx and liver cancer [5]. A lot of these cases are possible to be explained by the influence of carcinogenic substances from tobacco smoke but other probably have a genetic background. In the past 10 years, the molecular basis of many of these cancer syndromes have been unravelled with the advance of powerful genetic technologies, but on account of the lack of germ-line mutations, the position of lung cancer in the classification of CFS is still unknown.

COMMON HEREDITARY CANCERS

Cancer familial syndromes reflect an inherited predisposition to develop benign and malignant tumors. Most frequent in the classification are hereditary cancers of

the breast, ovary and colorectal. The identification of gene mutations that take place in individuals at high risk of these cancers has really improved our understanding of cancer predisposition [5, 32].

In most families affected with the breast and ovarian cancer syndrome or site specific ovarian cancer, genetic linkage has been found to the *BRCA1* locus on chromosome 17q21. Allelic deletion at the *BRCA1* locus in tumors from these linked family members invariably involves the wild-type chromosome, suggesting that *BRCA1* functions as a tumor suppressor gene. Rubin et al. suggested that germ-line *BRCA1* mutations occur only in small proportion of all ovarian cancers, but they are the most important prognostic and diagnostic factors [25]. Cancer associated with *BRCA1* mutation has a significantly more favorable clinical course. In Polish populations the entire coding region of *BRCA1* and *BRCA2* was screened for a presence of germ-line mutations by Gorski et al. Mutations were found in 53% of the menacing families (3 cases of cancer per a family) [8].

The molecular analysis techniques is based on the polymerase chain reaction (PCR) using genomic DNA, the reverse transcriptase PCR (RT-PCR) using mRNA or the sequencing of genomic DNA. The most precise molecular details are known for colon cancer. The *APC* gene of familial polyposis coli leads to the accumulation of numerous polyps, but the probability of transformation of the latter to cancer is low. Germline mutations of the *hMLH1* or *hMSH2* genes are the most frequent cause of the inherited susceptibility to colorectal and other epithelial cancers known as hereditary non-polyposis colorectal cancer [3, 10, 23]. Germ-line *Rb-1* gene mutations were found in families with isolated unilateral retinoblastoma patients. Germ-line mutations of *VHL* gene were identified in families with predisposition to cerebral, kidney, adrenal and eye cancers [35].

Emery et al. suggested that all families fulfilling criteria for hereditary cancers offered the possibility of genetic testing [5]. The strategies which support the integration of genetic medicine in primary care are needed to enable primary care practitioners to identify individuals at raised genetic risk and to reassure patients for whom genetic testing and increased surveillance offer early determining of threat, prevention or treatment [5, 14].

THE MOLECULAR GENETICS OF LUNG CANCER

With the development of molecular techniques the search for genetic alterations in lung cancer cells has resulted in the trials of a molecular description of cellular transformation. Most of these genetic changes occur in genes, which have a role in the control of cellular growth and development, meaning the activation of the protooncogens and inhibition of tumor suppressor genes. Frequently mutations of these genes induce the abnormalities in cell proliferation or in programmed cell death (apoptosis).

The most extensively studied oncogenes are the ras family genes (*H-K-N-ras*), *myc* family genes (*c-N-L-myc*) and *HER-2/neu* (*c-erB-2*) genes. Protein encoded by ras

genes acts in the signal transduction pathway from cell surface to the nucleus. Activated, mutated ras genes may stimulate uncontrolled growth autonomously [13]. *C-myc* gene transcriptionally controls the expression of different groups of genes. Its alterations result in cellular imbalance in the expression of genes that control both proliferation and apoptosis. The *c-erbB-1* and *c-erbB-2* oncogenes encode epidermal growth factor receptor and they are highly expressed in non-small cell lung cancer (NSCLC) cell lines. Small cell lung cancer (SCLC) cell lines demonstrated overexpression of *Bcl-2* gene, which encode mitochondrial oncoprotein in charge of apoptosis inhibition [11, 19, 20].

The best known tumor suppressor genes (*p53, RB, p16*) are inactivated when both alleles are mutated. The *p53* gene is located in chromosome 17p13. Product of *p53* gene stops cell cycle in G_1 phase. Cell cycle arrest enables repair of DNA damage or if the cells could not remove the injury, P53 protein directs the cells to apoptosis. Alterations in the *p53* pathway results in increased probability of neoplastic transformation and malignant progression [11, 12, 15, 26]. Protein Rb coded by *Rb* gene from chromosome 13q14 binds the sequestrates of transcription factors, which promote cell cycling and results in cell cycle arrest. Phosphorylation of *Rb1* gene by the CDK (cyclin-dependent kinase) causes the release of bound transcription factors that then stimulate cell division [11, 12].

Other important alterations include microsatelite instability (expansion or contractions in length of microsatelite sequences). These repetitions are highly polymorphic as a consequence of frequent germ-line mutations. The normal cell contains a DNA mismatch repair system which maintains the rates of spontaneous mutation at low levels. Mutations in *hMSH2* or *hMLH1* were identified as disorders in mismatch repairing genes in colon cancer [10]. Both SCLC and NSCLC are among the malignancies in which microsatellite instability is a relatively frequent event. Unstable replications of tandem repeats are localized in chromosome 3p and 2p [7, 11, 31].

The ras mutations (occurring most frequently in codons 12, 13 or 61 of *K-ras* gene) and *p53* mutations seem to be most profilic in NSCLC, while *myc* mutations are markers in SCLC patients. It has also been suggested that even in pre-invasive SCLC lesions the accumulation of genetic alterations is much higher than in NSCLC [21]. Gao et al. found *K-ras* mutations in 48% of lung cancer tissues. 67% of tumor tissues contained mutations in exons 7 and 8 of p53 gene. The frequency and the number of patients with *K-ras* and *p53* mutations between smokers and non-smokers was not different [6]. Kashii et al. described mutations of *K-ras* and *p53* genes in 22% of NSCLC lines and mutations of *p53* (43%) and *Rb1* (21%) genes in SCLC lines [13]. Johnson et al. report that more than one half of all lung cancer patients contain a mutation of the *p53* tumor suppressor gene [12]. No association appears to be between the presence of this mutation and patient survival. The oncogenes from the *ras* family were found to be mutated in approximately 20% of NSCLC in contrast to none in tumor tissues and tumor cell lines from patients with SCLC. Authors suggested that the presence of *K-ras* mutation was determined to be an adverse prognostic factor for survival in retrospective studies of patients with NSCLC. Mutation of *K-ras* is more common in

tumors from smokers than in non-smokers and in adenocarcinoma patients. Nishio et al. detected increasing expression of *H-ras* in cells from tumor tissues more than 80% of cases of adenocarcinoma, 39.5% of squamosus cell carcinoma, 21.4% of large cell carcinoma and only 15.4% of SCLC [22].

There are a lot of reports with the similar results concerning the protooncogen (*H-K-N-ras*, *c-N-L-myc*), mutations in cell lines and biopsy samples form lung cancer but constitutional mutations of this genes appear in a very small proportion in cells from other tissues. Gonzales et al. examined microsatelite alterations and *p53* mutations in tumor tissues, normal blood cells and plasma DNA. They certified that free plasma DNA with molecular alterations is present to a high degree in plasma DNA of SCLC patients and provide support for the potential use of this assay as a genetic marker for the early detection of lung cancer [7]. Minamoto et al. used cells from lavage fluid and sputum for molecular diagnosis of *K-ras* mutation and risk assessment. They reported that incidence of *K-ras* mutations was detected in 25–48% of lung cancer patients and suggested that molecular analysis is demonstrates promise in assessing susceptibility to, or risk of developing, sporadic cancers [18].

Another factor probably contributing to carcinogenesis is chromosomal imbalance and genetic instability. Typically in almost all SCLC and in proportion of NSCLC, majority of known deletions are located at the site of tumor suppressor genes, e.g. 3p14.2 (fragile histidine triad gene — FHIT), 3p21.3, 9p21 (p16) or 17p13.1 (p53). Other allelic losses in lung cancer were localized at 5q, 8p, 11p, 11q, 13q, 17q, 18q. Very important questions for clinical care and diagnosis of cancer is what kind of these alterations have a germ-line character [11, 31].

Methylation is one of the natural mechanisms, which leads to an inactivation of tumor suppressor genes (hypermethylation of CpG islands at the promotor regions). Methylated cytosine (5'methylcytosine) may undergo spontaneous deamination to tymine, resulting C → T transition. Such abnormal methylation may be associated with an overexpression of DNA methyltransferase [11].

The telomeres are tandem repeats of simple DNA sequence, which are located at the ends of the chromosome. Their function is to control the proper length of chromosome and cellular senescence. The length of telomeric repeats in normal cells is known to shorten progressively with each cell division. In telomeric regions is a critical threshold value of chromosomal shortening after which apoptosis ensures. The number of cell cycle repeats depends on the activity of telomerase — the reverse transcriptase that synthesizes telomeric DNA. In adult somatic cells the activity of telomerase is suppressed. High activity of this enzyme keeps telomere long in most cells from human cancers including lung cancer and makes possible the indefinite proliferation of cells [11, 27].

Cytogenetic methods, restriction fragment length polymorphism (RFLP) analyses, dot blot hybridization and sensitive PCR and RT-PCR, sequencing of genomic DNA, fluorescence hybridization *in situ* and biochemic methods have been used and they exhibited high specificity in detecting gene mutations and early detection of lung cancer. Molecular analysis focus at constitutional mutations that beside cigarette

smoke, environmental and dietary factors may also be involved in the genesis of lung cancer. Our knowledge of the genetic initiators and promoters of lung cancers, research for germ-line mutations as early diagnostic factors, should enable the development of intervention strategies that show great potential in individuals at high risk, especially smokers and exsmokers, and persons with FCS.

THE IMMUNOLOGIC RESPONSE IN LUNG CANCER

The defect in natural killer cell activity and intereferon and TNF-α response in human lung cancer were ascertained almost 20 years ago [17]. The influence of cytokiens on cytolitic activity of tumor infiltrating lymphocytes (TIL): cytotoxic T lymphocytes, large granular lymphocytes (LGL) and lymphokine-activated killer (LAK) is now well established [28]. The findings suggest that the local immune reaction favor the T helper type 2 (Th2) pathway instead of Th1 pathway. Local cellular immunity could be manipulated by interleukin-12 (IL-12), which exert effect on the helper T-cell pathway and the cytolytic activity of TIL [30].

IL-12 is recently discovered cytokine from antigen presenting cells (APC) with the potential for enhancing the cell-mediated immune responses to intracellular pathogens and tumors. IL-12 has an obligatory role for the generation of Th1 cells (producing IL-2 and INF-γ) and for optimal differentiation and activation of cytotoxic T lymphocytes and NK cells. Based on these unique features, IL-12 has been thoroughly examined and has demonstrated its ability to induce antitumor and antimetastatic effects [2, 28, 30, 33].

Immune-cytotoxic killing of the cells that are potentially harmful to the organism, such as virus-infected or tumor cells, are mediated by Fas ligand (FasL) and Fas. FasL is produced by T cells and NK cells activated by cytokines what in consequence induces apoptosis (programmed cell death) in target cell through the death receptor Fas/APO1/CD95 [4, 9]. Pitti et. al discovered a soluble decoy receptor, termed decoy receptor 3 (DcR3), that binds to FasL and inhibits FasL-induced apoptosis [24]. The *DcR3* gene localized in chromosome 20q32 was amplified in about half of 35 primary lung and colon tumors and *DcR3* mRNA was expressed in malignant tissue. Thus, certain tumors may escape FasL-dependent immune-cytotoxic attack by expressing a decoy receptor that blocks FasL [1, 34]. There are no reports about the expression of *DcR3* gene in other cells but it seems possible that the expression this gene increases in peripheral blood lymphocytes in neoplasms. Lee et al. found mutations of *Fas* gene in 7.7% patients with NSCLC in tumor cells and suggested that alterations of Fas gene may lead to the loss of its apoptotic function and contribute to the pathogenesis of some human lung cancers [16].

Flow cytometric analysis of subtypes of cytotoxic cells in peripheral blood and expression of cytokine receptors (IL-2R, IL-12R) and Fas antigen on their surface, determining of levels of cytokines in immunoenzymatic techniques and estimation of expression of *DcR3* mRNA in tissues by northern blotting or RT-PCR could be helpful for prevention of lung cancers in persons with FCS as the supplement methods for

genetic analysis. Some of these markers might be useful for monitoring of the response to the therapy, including early detection of tumor recurrence to allow curative therapy and rapid detection of treatment failure to allow the change of the regiment. The study of these markers may also lead to a better understanding of the biological characteristics of lung cancer.

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STRESZCZENIE

Każdego roku na świecie notuje się prawie milion nowych zachorowań na różne postaci raka płuc. Przyczyn wzrostu liczby chorych należy dopatrywać się nie tylko w czynnikach środowiskowych, przede wszystkim w szkodliwym działaniu dymu tytoniowego, ale również w zaburzeniach genetycznych. Zanotowano wielką różnorodność mutacji genów sprawujących kontrolę nad proliferacją i apoptozą komórek ulegających procesowi nowotworowemu. Badania skupiły się przede wszystkim nad nieprawidłową funkcją protoonkogenów i genów hamujących rozwój guzów. Coraz więcej materiałów dotyczy genetycznych nieprawidłowości wpływających na zaburzenia odpowiedzi immunologicznej w chorobach nowotworowych. Istnieje kilkanaście zespołów rodzinnej skłonności do wystąpienia nowotworów, w których badania genetyczne znajdują praktyczne zastosowanie we wczesnej diagnostyce i prewencji. W rakach płuc nadal nie znaleziono typowych markerów genetycznych, których analiza umożliwiłaby objęcie szczególną kontrolą osób narażonych na zachorowanie. W pracy zostały przedstawione czynniki o potencjalnym znaczeniu prognostycznym w raku płuca i niektóre sposoby ich praktycznego wykorzystania.