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review

Molecular biology of the lung cancer

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Background. *Lung cancer is one of the most common malignant diseases and leading cause of cancer death worldwide. The advances in molecular biology and genetics, including the modern microarray technology and rapid sequencing techniques, have enabled a remarkable progress into elucidating the lung cancer ethiopathogenesis.*

Numerous studies suggest that more than 20 different genetic and epigenetic alterations are accumulating during the pathogenesis of clinically evident pulmonary cancers as a clonal, multistep process. Thus far, the most investigated alterations are the inactivational mutations and losses of tumour suppressor genes and the overexpression of growth-promoting oncogenes. More recently, the acquired epigenetic inactivation of tumour suppressor genes by promoter hypermethylation has been recognized. The early clonal genetic abnormalities that occur in preneoplastic bronchial epithelium damaged by smoking or other carcinogenes are being identified. The molecular distinctions between small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), as well as between tumors with different clinical outcomes have been described. These investigations lead to the "hallmarks of lung cancer".

Conclusions. *It is realistic to expect that the molecular and cell culture-based investigations will lead to discoveries of new clinical applications with the potential to provide new avenues for early diagnosis, risk assessment, prevention, and most important, new more effective treatment approaches for the lung cancer patients.*

Key words: lung neoplasms-genetics; genes, tumor suppressor

Introduction

Lung cancer is one of the most common malignant diseases and leading cause of cancer death worldwide with estimated more than 1.3 million new cases each year.[1] The lung cancer incidence and mortality have risen into epidemic proportions in Western world during the 20th century.[2] The majority of lung cancer patients is inoperable or has disseminated disease at the time of diagnosis and displays a remarkable insensitiveness to chemotherapeutics and radiation therapy.[3] Over 85% of these patients eventually die from disseminated disease during the first 5 years and this extreme mortality has not changed significantly during the last three decades. Despite diagnostic and

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therapeutic improvements, the 5-year survival rate has barely increased from 7 to 14% since 1970-thies to the present. Moreover, the lung carcinoma is accounting for nearly 29% of all cancer-related deaths in both genders, that exceeds the sum of the next three leading causes of death due to breast, colon, and prostate cancer.[4]

It is believed that smoking is the primary etiologic agent in more than 80% of lung cancer patients.[5] The other risk factors include, but are not limited to, passive smoking, exposure to environmental pollutants, occupational exposure to chemicals (arsenic, asbestos, chromium, nickel and vinyl chloride) and to the natural radioactive gas radon.[2] Genetic predisposition, especially polymorphisms of the tumor suppressor genes and the allelic variants of the genes involved in detoxification, are implicated into the susceptibility to the disease.[6]

Based on the histopathological classification (WHO, 1977), lung cancer is divided into two main types: non–small cell (NSCLC) and small cell lung cancer (SCLC), which are delineated by their biological and clinical features. Furthermore, NSCLC consists of several subtypes, predominantly adenocarcinoma, squamous-cell carcinoma, and large-cell carcinoma. SCLC is a distinct clinicopathological entity with neuroendocrine pathophysiologic features and characteristic microscopic morphology.[7] SCLC represents roughly 20% of all pulmonary cancers. The histologic distinction between NSCLC and SCLC is clinically extremely important. There are considerable differences between those two groups in both, therapeutic approach and prognosis of the disease. Recently, molecular classification of lung carcinomas has been made using mRNA expression profiling by microarray technology.[8-10]

Molecular biology of lung cancer

It is generally accepted that the pathogenesis of human cancer involves the accumulation of multiple molecular abnormalities over time. Those alterations lead to acquired cellular capabilities that can be classified in the following six functional sets: a) self-sufficiency in growth signals due to mutations in proto-oncogenes, b) insensitivity to antiproliferative signals as a result of mutations affecting the tumour suppressor genes, c) evading of apoptosis by up-regulation of antiapoptotic or down-regulation of proapoptotic molecules, d) limitless replicative potential due to the activation of telomerase, e) sustained angiogenesis and f) capability for tissue invasion and capability for dissemination into distant sites (metastasis).[11] Those molecular alterations can occur at the level of gene up-regulation or down-regulation, DNA sequence changes (point mutations), loss of heterozygosity (i.e., deletion of one copy of allelic DNA sequences), DNA segment amplification or whole chromosome gains or losses with the simultaneous genomic instability and alterations in microsatellite DNA.[12,13]

The advances in molecular biology and genetics, including the modern microarray technology and rapid sequencing techniques, have enabled a remarkable progress into elucidating the lung cancer ethiopathogenesis. Numerous studies suggest that more than 20 different genetic and epigenetic alterations are accumulating during the pathogenesis of clinically evident pulmonary cancers as a clonal, multistep process.[14-16] Thus far, the most investigated alterations are the inactivational mutations and losses of tumor suppressor genes and overexpression of growth-promoting oncogenes. More recently, the acquired epigenetic inactivation of tumor suppressor genes by promoter hypermethylation has been recognized. The early clonal genetic abnormalities that occur in preneoplastic bronchial epithelium damaged by smoking or other carcinogenes are being identified. The molecular distinctions between SCLC and NSCLC, as well as between tumors with different clinical outcomes have been described.

These investigations lead to the "hallmarks of lung cancer".[3] It is realistic to expect that the molecular and cell culture-based investigations will lead to discoveries of new clinical applications with the potential to provide new avenues for early diagnosis, risk assessment, prevention, and most important, new more effective treatment approaches for the lung cancer patients.

Growth stimulation by oncogenes

Protein-tyrosine kinases (PTKs) are vital regulators of intracellular signal-transduction pathways that mediate development and cell-to-cell communication. Their activity is normally firmly controlled and regulated. Disturbances in the PTK signaling resulting from mutations and other genetic alterations contribute to the malignant transformation. A number of growth factors and their receptors are expressed by lung cancer cells or their neighboring stromal cells, thus producing autocrine or paracrine growth stimulation loops. Several are encoded for by proto-oncogenes which become activated in the course of the lung cancer development.[3] The overexpression of cell cycle regulatory proteins such as **cyclin D1**,[17] **cyclin E**,[18] and **cyclin B1**,[19] enhance the cell proliferation, decrease the cellular apoptotic potential and are commonly found in NSCLC tumor specimen.

Epidermal growth factor receptor (EGFR), also called ErbB-1, is the member of a subfamily of closely related proteins. After ligand-binding, the intracellular tyrosine kinase domain of the EGFR receptor is activated and undertakes autophosphorylation, which initiates a cascade of intracellular events. A downstream signaling pathway involves the activation of p21-Ras and mitogen-activated protein kinases (MAPKs). EGFR signaling is critical for the normal cell proliferation, but its deregulation is crucial for cancer pathogenesis, neoangiogenesis, metastasis, and apoptosis inhibition.[20] EGFR is overexpressed in the advanced NSCLC, and is associated with the poor survival and resistance to chemotherapeutic agents, including cisplatin. The results of different studies investigating the prognostic value of EGFR expression in lung cancer are contradictory.[3] However, since EGFR expression is clearly involved in the lung cancer pathogenesis, this molecule is an attractive target of different therapeutic approaches.[21] Few EGFR inhibitors (CP358774, ZD1839-Iressa and OSI774) are under intensive clinical trials in lung cancer patients.[3]

HER-2/neu (ErbB-2) gene is located on chromosome 17p21 and encodes for a 185-kDa transmembrane glycoprotein ($p185^{HER\text{-}2/neu}$) that has high homology with EGFR. *HER-2/neu* is overexpressed in about 30% of NSCLCs, particularly in adenocarcinomas and is associated with multiple drug resistance phenotype and high prevalence of metastases.[3] A point mutation resulting in the substitution of the amino acid residue 664 from valine to glutamic acid is commonly found, and this mutation contributes to the malignant transform of affected cells. Alterations and amplifications of *HER-2/neu* gene have been reported in NSCLC.[20] Chemotherapy combined with trastuzumab (Herceptin), a monoclonal antibody against the HER2/neu receptor is now under clinical trials.[3]

MYC proto-oncogene belongs to a family of related genes (*c-MYC, N-MYC, L-MYC*) which encode transcription factors that activate genes involved in the growth control and apoptosis. The MYC phosphoproteins are localized in the nucleus.[22] The transcriptional regulation by MYC proteins is mediated by heterodimerizing with partner proteins such as MAX, MAD or MX11.[23] MYC-MAX heterodimer binds to specific DNA sequences named E-box elements in the neighborhood of promoters of downstream target genes and activate their transcription. Histone acetylase

is activated and leads to alterations in chromatin structure, which, in turn, modulate the gene transcription. On the other hand, the MYC-MAX complex represses a transcriptional activation. MAX can bind MAD and MX11 proteins to repress transcription, antagonize MYC, and promote cellular differentiation.[20] The molecular abnormalities involving the *MYC* genes or their transcriptional deregulation were found to be an important molecular mechanism in the pathogenesis of human lung cancers.[23] The most frequent abnormality involving *MYC* members in lung cancer is gene amplification or gene overexpression without amplification. The overexpression of a *MYC* gene, with or without amplification, occurs in 80 to 90% of SCLCs.[22] In contrast to SCLC, the amplification of the *MYC* gene occurs only in approximately 10% of NSCLC samples. However, *MYC* overexpression without *MYC* gene amplification occurs in over 50% of NSCLC investigated specimens.[22] *MYC* gene overexpression has been identified to be a late event in lung cancer pathogenesis in the vast majority of SCLCs.[20] Lung tumor cell lines established from metastatic tumors have a high frequency of *MYC* amplification, and this probably explains the correlation of *MYC* amplification with a poor clinical prognosis.[24] The antisense oligonucleotides therapy models directed at downregulating *MYC* expression show encouraging results in cell culture.[3]

The dominant ***RAS* proto-oncogene** is extremely important for the transduction of the growth-promoting signals from the membrane to the nucleus and consequently for the cellular proliferation. The *RAS* family of genes includes: the *HRAS* gene (homologous to the oncogene of the Harvey rat sarcoma virus), the *KRAS2* gene (homologous to the oncogene of the Kirsten rat sarcoma virus) and the *NRAS* gene (initially cloned from human neuroblastoma cells). The *RAS* genes code for four highly homologous 21 kDa proteins called p21 anchored to the inner side of the plasma membrane, where they can effectively interact with their upstream activators and downstream targets. In active state RAS proteins binds to guanosine triphosphate (GTP) and through the intrinsic GTPase activity and conformational change of RAS, the GTP hydrolyze to guanine diphosphate (GDP) and after interacting with its substrate Raf1, RAS returns to the inactive state. The cell proliferation signal is subsequently transmitted by a cascade of RAS-dependent kinases, activating the MAPK, which translocate to the nucleus and initiate transcription factors.[20] This signal transduction pathway is sometimes called SOS-Ras-Raf-MAPK mitogenic cascade.[11] In malignant cells, the point mutation in the RAS gene can make the RAS protein defective in the intrinsic GTPase activity that becomes locked into the growth stimulatory GTP-bound form, constantly sending the signal stimulating cell proliferation signals to the nucleus.[25] *RAS* mutations are very rare or absent in SCLC, but can be identified in 15-20% of NSCLC. Up to 50% of the lung adenocarcinomas carry *RAS* mutations,[26] usually affecting codon 12 of *KRAS* (85% of cases), and rarely codon 13 of *HRAS*, or codon 61 of *NRAS* gene.[23] The majority (up to 70%) of these mutations are G→T transversions that are induced by benzopyrene diethyloxide (BPDE), nitrosamines and other DNA adducts-forming agents that are present in the tobacco smoke. It is believed that this is the reason for the correlation between smoking history and the frequency of *KRAS* mutations in NSCLC samples which are associated with poor prognosis.[27] Few clinical trials are conducted: using vaccination with mutant KRAS peptides, by suppression of the mutant *RAS* gene using antisense oligonucleotides, or by inhibition of the farnesylation of the RAS protein that is necessary for its activation.[3]

The distinguishing feature of SCLC tumors is the production and release of a broad range of neuropeptides from the neoplastic cells.

Angiotensin, bombesin, insulin-like growth factor 1, vasopressin, serotonin, and substance P are among the best studied signal molecules released by SCLC cells.[28] These peptides act as ligands for high-affinity receptors on the tumor cell surface, and their binding consequently activate the G-protein coupled receptors enabling a further intracellular transmission of the proliferative signal. By this, SCLC cells are self-stimulating the growth by autocrine and paracrine manner.

Insensitivity to anti-growth signals: tumor suppressor genes

Tumor suppressor genes (TSG) play a critical role in cell's antiproliferative circuitry and are also involved in the cellular response to DNA damage and consequent reparation processes. There is a frequent loss of tumor suppressor genes during the pathogenesis and progression of lung cancers, as in many epithelial cancers. The inactivation of the tumor suppressor genes occurs by loss of one allele from the chromosomal locus, termed loss of heterozygosity (LOH) and damage to the other allele by gene mutation or the epigenetic hypermethylation of its promoter. The chromosomal regions that where found to be most frequently affected by LOH in lung carcinomas are 1p, 3p, 4p, 4q, 5q, 8p, 9p (p16 TSG locus), 9q, 10p, 10q, 13q (*RB*-retinoblastoma locus), 15q, 17p (p53 locus), 18q, 19p, Xp, and Xq.3 The allelic loss at several loci on the chromosome arm 3p is one of the most frequent and earliest genetic events in lung cancer pathogenesis found in up to 96% of carcinomas and 78% of preneoplastic bronchoepithelial lesions.[29] The high frequencies of LOH and frequent homozygous deletions found in many lung cancer cell lines and tumor samples suggest that few potential tumor suppressor genes reside at this chromosome region.[23] Moreover, the frequency and size of the allelic loss of 3p correlate with the severity of histopathological preneoplastic/preinvasive grades. There are a number of other candidate tumor suppressor genes located at 3p and their allelic loss may probably be the earliest acquired genetic abnormality in the lung cancer pathogenesis.[3,30]

FHIT is a tumor-suppressor gene located at 3p14.2, coding for a dinucleoside 5′, 5‴-P1-P3-triphosphate hydrolase protein product (often denoted as pFHIT). The loss of the gene results in the accumulation of diadenosine tetraphosphate, thus stimulating DNA synthesis and cell proliferation. A decreased expression of *FHIT* has been found in 49% of NSCLC specimen by immunochemistry. pFHIT expression is significantly reduced in a large number of early-stage NSCLC and preneoplastic lesions in chronic smokers. The association between cigarette smoking and pFHIT expression suggests a role for *FHIT* in the initiation of smoking-related lung carcinogenesis.[20] It was demonstrated that the reintroduction of wild-type *FHIT* inhibits lung cancer in vitro growth and in vivo tumorigenicity in nude (athymic) mice.[23]

The ***RARβ.*** **(retinoic acid receptor beta) gene,** located at 3p24 is a strong TSG candidate. Low or absent *RARβ.* expression was detected with high frequency in lung cancer cell lines and primary lung tumours.[23] It appears to result from the aberrant promoter methylation of the *RARβ* and was observed in approximately 40% of primary SCLCs.

The ***TP53 tumor-suppressor gene (p53)*** is located at chromosome arm 17p13.1 and encodes a 53 kDa nuclear protein that acts as a DNA-binding, sequence-specific transcription factor that activates the expression of genes engaged in promoting growth arrest in the G1 phase or cell death in response to the genotoxic stress.[31] Thus, p53 has a role of "guardian of the genome", maintaining the genome integrity during the cellular stress from DNA damage, hypoxia, and activated oncogenes. Also, p53 prevents cells with damaged DNA from undergoing mitosis

when they enter the G_2 phase. p53 blocks cells at the G_2 checkpoint, at least partially, by inhibition of cdc2, the cyclin-dependent kinase required to enter mitosis. The ability of p53 to inhibit cellular proliferation or to induce apoptosis is suppressed by *HDM2* protein product, the human homologue of the murine double minute 2 *(MDM2)*. This protein blocks p53 regulation of target genes and enhances its proteasome dependent degradation.[31] On the other hand, p53 upregulates the expression of *HDM2* by directly binding and activating the *HDM2* promoter and thus p53 is downregulating its own expression. This autoregulatory loop keeps p53 at virtually undetectable levels in normal cells.[3] Missense mutations (mainly G→T transversions) clustered in the middle of the gene at codons 157, 245, 248, and 273 abolishes its tumor suppressing activity and extend the p53 mutant protein half-life that can be easily detected by immunohistochemistry. The *p53* gene mutations in lung cancer have been extensively investigated and were found that *p53* is inactivated in 75% of SCLCs and about 50% of NSCLCs and the frequency of mutations correlate with cigarette smoking.[20] It is intriguing that the mutations at codon 157 appear to be unique to pulmonary carcinomas, while codon 248 and 273 hot spots mutations occur in other cancers, *e.g.*, colon, liver, and prostate.[22] Nonsmokers who develop lung cancer have a completely different, almost random grouping of *p53* mutations.[22] Although the prognostic role of *p53* mutations in NSCLC p53 is still under debate, their presence influences the clinical response to cisplatin-based chemotherapy and radiotherapy.[3]

The ***RB* tumor-suppressor gene** is located on chromosome 13q14 and its protein product is a nuclear phosphoprotein initially identified in childhood retinoblastomas. RB protein cooperates with p53 in the regulation and control of cell cycle progression, the transcriptional level, and the equilibrium between the cell differentiation and proliferation. The phosphorylation status of the RB protein and its interaction with transcription factor E2F is most important for the regulation of G0/G1 cell cycle transition. When RB is dephosphorylated, it suppresses the G1 to S phase transition.[32] During G1 phase, cyclin D1 is associated with cyclin-dependent–kinases CDK2 and CDK4 that results in phosphorylation and activation of RB. Hypophosphorylated RB binds the E2F transcription factor, thus blocking the transcription of genes regulating the cell cycle. On the contrary, when RB is phosphorylated, E2F dissociates and activates the transcription, thus facilitating S phase entry.[23] Abnormalities of the *RB* gene in lung cancer include deletions, nonsense mutations, pathogenic splicing variations and chromosomal deletions. The disruption of the pRb pathway releases E2Fs allowing cell proliferation to proceed and making the cell insensitive to antigrowth factors that normally function to control a transition through the G1 phase of the cell cycle.[11] More than 90% of the SCLC and 15-30% of the NSCLC neoplasms have abnormal or no *RB* expression.[22] Although *RB* plays an important role in pulmonary cancer pathogenesis, pRB status has no prognostic significance in NSCLC patients.[20]

***PTEN* (Phosphatase Tensin Homolog Deleted on Chromosome Ten)** gene is located at chromosome 10q23 encodes a lipid phosphatase which dephosphorylates PIP3 and posses tumor suppressor activity *in vitro* and *in vivo*. Mutations or deletions of the PTEN gene have been found in a few lung cancer cell lines and tumor samples.[23]

Transforming growth factor-β (TGF-β) is multifunctional protein that inhibits the proliferation of many epithelial cells through binding with a set of cell receptors. It is a checkpoint inhibitor involved in the cell cycle regulation, causing cells to cease proliferation and arrest in G_1.[22] The reduced levels of TGF-β expression was found in NSCLC

samples by immunocytochemical staining studies.

Another candidate TSG on chromosome 10q25-26 is ***DMBT1.*** It is frequently down regulated and occasionally homozygously deleted in lung cancer.[23] The overexpression or activation of **insulin-like growth factor I receptor *(IGF-IR)*** has been observed in many human cancers including pulmonary carcinomas. The ***p16^{INK4}*** (also termed ***CDKN2A***) is a tumor-suppressor gene located on chromosome 9p21 and codes for two proteins translated by alternative mRNA splicing: α-transcript that is translated into p16 (p16^{INK4}) and β-transcript that is translated into p14^{ARF} protein. p16 protein that is part of the p16-cyclin D1-Cdk4-RB pathway.[32] p16 regulates cell-cycle progression through a G_1/S restriction point by inhibiting CDK4 and CDK6/cyclin D-mediated phosphorylation of pRB.[20] The disruption of *p16* function results in inappropriate hyperphosphorylation and, therefore, inactivation of pRB. The overexpression of the E2F transcription factor upregulates *p16* expression and inhibits cyclin D-dependent kinase activity, suggesting the presence of a feedback loop. **p14^{ARF}** protein binds to and stabilizes HDM2 (MDM2 homologue), increasing its availability of wild-type p53. The loss of p14^{ARF} or p53, which are common genetic lesions in lung cancer, permits an amplified *MYC* free opportunity for the cell proliferation and transformation. p14^{ARF} appears to bridge a gap between oncogenic signals and p53 whereby p14^{ARF}-induced activation would be critical to move the compromised cell toward apoptosis.[22, 31] The expression of p16^{INK4} gene in NSCLCs is frequently altered by abnormal promoter methylation (25% of cases) and homozygous deletions or point mutations (10%-40%).[23] It was found that the disturbances in both, the *p16/pRb* and *p53* pathways are essential for the enhanced proliferation of NSCLC cell lines. There is an inverse relation between p16 and Rb in pulmonary carcinomas: *Rb* is mutated and p16 is intact in SCLC, while p16 expression is disrupted and *Rb* is usually intact in NSCLC.[22] **p19^{ARF}** binds to the MDM2-p53 and prevents p53 degradation. The loss of p19^{ARF} is more frequent in lung tumours with neuroendocrine features.[23, 31]

Evading apoptosis

Apoptosis or programmed cell death is a genetically controlled process that is essential for tissue remodeling during embryogenesis and for the maintenance of the homeostatic balance of cell numbers during adult life. A deregulation of cell death pathways is implicated in tumor initiation, progression, and drug resistance in many human cancers and is one of the hallmarks of cancer.[11, 33] Two major intracellular apoptosis signaling pathways can lead to programmed cell death, the mitochondrial pathway (intrinsic) and the death receptor (extrinsic) pathway. Mediated by a cascade of caspase activations and other mediator proteins, both pathways finally lead to the proteolytic cleavage of a variety of cellular proteins, induces DNA fragmentation and numerous morphological changes that are characteristic of cells undergoing apoptosis. Key genes that regulate apoptosis include the *p53* tumour suppressor gene and the Bcl-2 gene family. Simplified, the *BCL-2* family members are major regulators of the apoptotic process, whereas caspases are the major executioners.

Bcl-2 (B-cell lymphoma-2) gene was the first oncogene found to function through the production of an inhibitor of apoptosis. The *bcl-2* gene family consists of more than 15 members, which either promote or inhibit the apoptosis.[34,35,36] The bcl-2 gene is located on chromosome arm 18q21 and the BCL-2 protein product is localized within the outer mitochondrial membrane, endoplasmic reticulum and the nuclear envelope, where it exerts anti-apoptotic effect within many cell types.[34] Following the apoptotic stimulation, pro-

apoptotic proteins are activated through post-transcriptional modifications or changes in their conformation. BCL-2 protein forms heterodimers with proapoptotic BCL-2 family members, leading to their inactivation. In addition, BCL-2 proteins may interfere with critical steps during the integration of proapoptotic signals at the level of mitochondria, thereby abrogating cytochrome-C release. BAX is a BCL-2-related protein which promotes apoptosis and is a downstream transcription target of p53. BCL-2 protein heterodimerizes with BAX consequently inhibits apoptosis. Tumor cells often escape apoptosis as the normal physiological response when challenged by cellular and DNA damage. BCL-2 overexpression, detected by immunohistochemistry, was found in 75%-95% of SCLC tumors, 25%-30% of the squamous cell carcinomas and in 10% of adenocarcinomas.[37] The significantly higher incidence of *bcl-2* overexpression in SCLC is unexpected as these tumors are more sensitive to chemotherapeutic agents that induce an apoptotic response.[3] Interestingly, the expression of BAX and BCL-2 proteins is inversely related in neuroendocrine cancers. Namely, high BCL-2 and low BAX expression occurs in most SCLC tumors which are also mostly *p53* deficient.[3] The significance of the *bcl-2* expression in lung cancer for the overall survival is controversial, but *bcl-2* expression was found to be associated with a better prognosis in NSCLC patients that may be associated with the lower tumor vascularization.[20,38]

Limitless replicative potential - telomeres and telomerases

Telomeres are specialized heterochromatin structures at the end of each chromosome that serves as protective caps and plays a role in the maintaining chromosome integrity, reversibly represses the transcription of neighboring genes and prevents the end-to-end fusion or degradation of the chromosomes.[39] Due to the inability of the conventional DNA polymerases to replicate the 5′-end of linear DNA, telomeres shorten during each cell division in the normal human somatic cells. This phenomenon is known as an end-replication problem. This shortening does not produce the loss of the essential genes in which each of the 46 human chromosomes is capped with long repeats of non-coding DNA sequences named telomeres. The human telomeres consists of highly repetitive DNA of tandem sequences TTAGGG)*n*.[40,41] It has been calculated that roughly 50–100 bp are lost with each round of cell division.[42] Human cells are estimated to have the potential to undergo on average 50–70 divisions. At this point the cell growth arrests and enters senescence. A dozen of telomeric proteins are needed to hide the telomeres from the cellular machinery that would normally treat the end of a linear DNA molecule as a broken strand needing repair.[43] The key telomeric DNA binding proteins are the telomeric repeat binding factors, Tankyrase, heterogeneous nuclear ribonucleoproteins and few other functionally related proteins. The physiologic maintenance of the telomere requires complex interactions among these proteins, telomeric DNA, and other cellular factors. Telomere integrity is also essential for the chromosome numerical and positional stability and the telomere shortening facilitates the evolution of cancer cells by promoting chromosome end-to-end fusions and the development of aneuploidy. The inhibition of telomerase in immortal cancer-cell lines by genetic or pharmacological methods results in telomere shortening and eventually halts cell proliferation.[44]

Telomerase is a specific ribonucleoprotein enzyme complex that elongates and maintains the preexisting telomeres of eukaryotic chromosomes, using an intrinsic RNA molecule as a template and thus is extending the number of divisions the cell may undertake.[45] Telomerase holoenzyme contains two main

components that are essential for the activity: hTERT subunit (RNA-directed DNA polymerase, *i.e.* reverse transcriptase, EC 2.7.7.), and hTR, 451-nt RNA chain that serves as a template. The enzyme complex also contains many proteins necessary for the full enzymatic activity that are collectively named as telomerase-associated proteins. The gene for the telomerase catalytic subunit *hTERT* is more than 37 kb in length and consists of 16 exons.[46] The telomerase activity is absent in the majority of normal cells in adult organisms, but is increased during the development and neoplasia.[47] Since over 90% of human neoplastic cells have increased telomerase activity, it is now generally accepted that this is a one of the cancer hallmarks and extremely frequent and consistent cancer-associated molecular abnormality. Generally, the telomerase expression in malignant tumors is determining the capacity for the unlimited proliferation and thus immortality. A high telomerase activity was detected in almost 100% of SCLC and 80% of NSCLC samples using a PCR-based telomeric repeat amplification protocol (TRAP assay). A high telomerase activity in primary NSCLC was found to be associated with the increased cell proliferation rates and advanced pathologic stage.[48] Recently, the telomere shortening was found to be an early molecular abnormality in bronchioepithelial carcinogenesis, preceding telomerase expression and p53/Rb inactivation that occurs in most high-grade preinvasive lesions.[49] Since the telomerase activity is associated with malignant growth, it is a marker for lung cancer detection, and a important target for novel therapeutic approaches.[23]

Tumor angiogenesis

New blood vessel growth (neovascularization or neoangiogenesis) is required for tumors to sustain and grow beyond 3 mm in diameter and for metastasis. Different inducers and inhibitors regulating endothelial cell proliferation and migration are involved in the process of angiogenesis. Growth factors that have been shown to stimulate angiogenesis include vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived endothelial cell growth factor (PD-EGF) and platelet-derived growth factor (PDGF).[3,23] The productions of angiogenesis factors apparently influence the clinical outcome of lung cancer patients. Namely, the VEGF levels in plasma are correlated with the degree of angiogenesis in NSCLC and the VEGF expression was found to be associated with the decreased overall and disease-free survival in NSCLC patients.[50] Immunochemical studies demonstrated that bFGF is a prognostic indicator in lung adenocarcinoma, since the 5-year survival rate was significantly lower for bFGF positive patients and the more aggressive clinical behavior was associated with up-regulation of PDGF.[23] In a few clinical trials, impressive results were achieved by targeting VEGF with a "humanized" monoclonal anti-VEGF antibody. Unfortunately, unexpected bleeding from large necrotic lung neoplastic masses occurred in the initial trials, but this should be approachable by a more careful patient selection.[3]

Tissue invasion and metastasis

Molecular mechanisms that lead to the complex ability of the primary lung cancer cells to invade the adjacent tissue and to disseminate to the distant organs of the patient's body are mainly unknown.[3] This process involves degradation of the basement membrane, invasion of the surrounding stroma and the blood or lymphatic vessel, ability to growth without adhesion, angiogenesis, cell proliferation, and migration.[11] Few different genes and their protein products are identified to be important for the process of tissue invasion

and metastatic capability of the neoplastic cells.

E-cadherin is a cell adhesion molecule that is universally expressed on epithelial cells. During the pathogenesis of most epithelial cancers, E-cadherin function is lost by the mutational inactivation of the E-cadherin or **β-catenin** genes, as well as by the transcriptional repression, or enhanced proteolysis. This results in reduced E-cadherin-mediated cell-cell adhesion and enables the malignant cells to invade the tissues and to enter the blood or lymphatic vessels.[51] Therefore, E-cadherin gene is sometimes referred to as the "suppressor of invasion" gene.[52] It was demonstrated that E-cadherin loss in lung cancer is associated with the increased metastasis capability.[53] A degradation of the basal membrane and of the extracellular tissue matrix by proteases is very important for the local invasiveness and blood or lymphatic metastasis.

Matrix metalloproteinases (MMPs) are members of the family of zinc-containing proteolytic enzymes that facilitate the tumor invasion, the metastatic capabilities, and the tumor-related angiogenesis. Conversely, matrix metalloproteinase inhibitors (MMPIs) have been shown to inhibit tumour growth and dissemination in preclinical models. It is therefore not clear why not all lung cancers express the MMPs and there are conflicting reports about the prognostic importance of MMPs expression in lung cancer.[54]

It was found that **CRMP-1,** a protein that mediates the effect of collapsins, has reduced the expression in more aggressive and metastatic lung cancer samples.[55] This down-regulation is believed to enhance the cell migration ability, which is important for the process of metastasis. CRMP and other members of the collapsin/semaphorin protein families might control the cell's movement.[56]

Laminins and **integrins** are proteins involved in the adjacent tissue invasion through the basement membrane and further spread of the lung cancer cells. The reduced expression of laminin α chains (α3 and α5) in lung neoplastic tissue might result in the basal membrane fragmentation necessary for the cancer cell invasion.[57] Changes in the integrin expression are found in metastatic cells in many human neoplasms, including the lung cancer.[11] Recently, a study conducted by Manda and collaborators, identified that the ***LAMB3*** gene (coding for the laminin β3 chain, a component of laminin-5) was expressed only in NSCLC cells and not in SCLC tumor cells.[58] In the same study, the **α6β4** integrin, the specific laminin-5 binding receptor, was expressed only in NSCLC cells but not in SCLC cells. This suggests that laminin-5 might be a critical microenvironmental factor for the growth of NSCLC tumours.[58]

Overview of the molecular abnormalities in lung cancer pathogenesis

The model of lung cancer pathogenesis is depicted on the Figure 1 and was developed based on the previous studies.[59] The carcinogens from the tobacco or other environmental pollutants lead to the loss of the 3p21.3 allele in thousands of cells on different sites of the respiratory epithelium. Later, the tumor suppressor genes located in the 3p21.3 chromosome arm become haplo-insufficient. The next hit occurs in genes that are critical for the cell proliferation, such as *RB, p53, p16* or other genes either by the mutational inactivation or by the promoter hypermethylation. That permits a clonal outgrowth of the initially transformed cells. Some authors suggest that the molecular pathogenesis differs significantly between SCLC and NSCLC main tumor types.[30] It is proposed that during the pathogenesis of the SCLC neoplastic cells arise directly either from normal or hyperplastic epithelial cells without passing through characteristic preneoplastic intermediate pathological stages (parallel theory of lung cancer

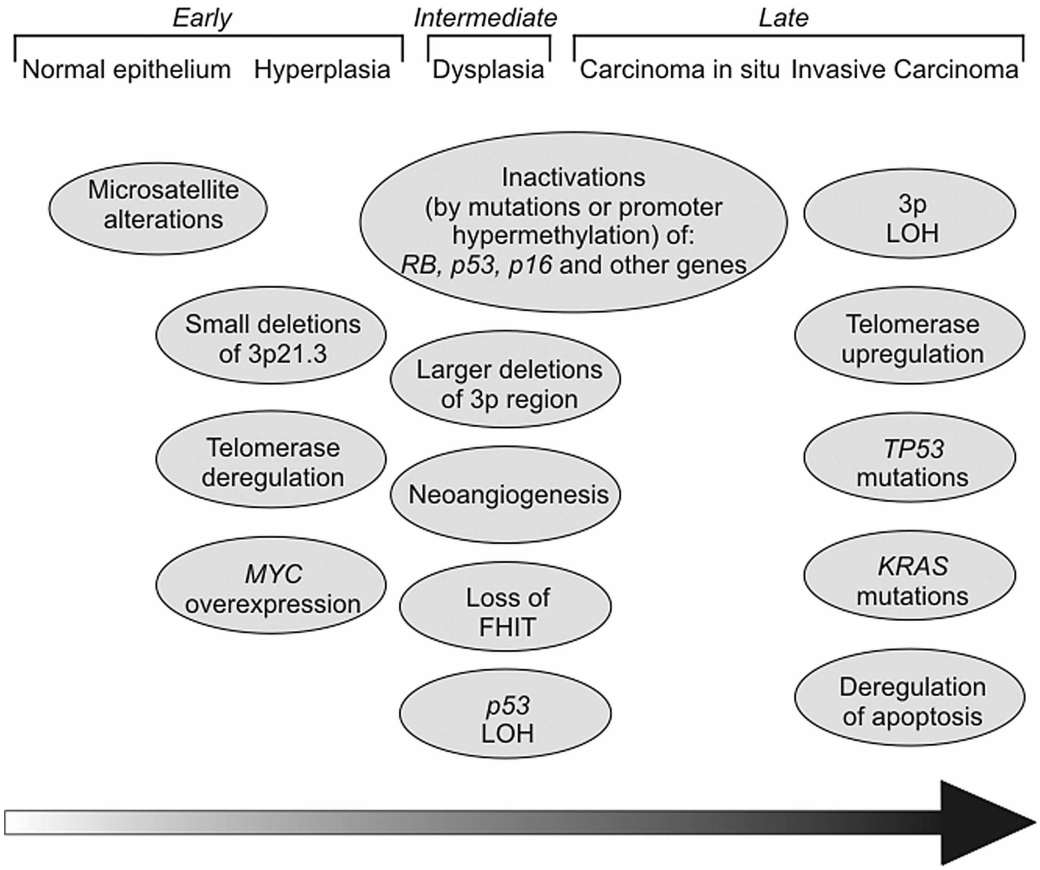

Figure 1. Main molecular abnormalities occurring during lung cancer pathogenesis (according to References 59 and 22).

pathogenesis). On the contrary, the NSCLC pathogenesis is accompanied with sequential morphological changes (sequential theory).

Conclusions

Recent progress into the elucidation of the molecular genetic abnormalities involved in the lung cancer has been achieved using modern technologies for the mutation detection as well as for the gene expression quantitation using microarray techniques. A precise characterization of those events and their association with the clinical and pathological types of the lung cancers are expected to result in the clarification of the pathogenesis of this complex disease and would led to the advance of novel molecular approaches for the early diagnosis and therapy of the pulmonary carcinomas.

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