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Human Lung Cancer: Cancer-Causing Genes and Environmental Factors.

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Abstract

Recently much progress was achieved in understanding the genetic etiology of lung cancer. This progress, combined with the well understood role of environmental causative risk factors, is leading to the development of new diagnostic and therapeutic strategies to combat and prevent the lung cancer epidemic. Environmental agents such as tobacco smoke, radon, radiation, air and industrial pollutants are now well known to act by damaging cancer-causing genes, thereby initiating transformation and clonal development of transformed cells in the lung epithelium. We review the basic facts and concepts in lung cancer pathogenesis describing involved cancer-causing genes, clonal origin and development of initiated, pre-malignant cells into preinvasive and invasive tumors and novel strategies for early diagnosis and treatment of lung tumors.

Introduction

Despite the enormous progress achieved in molecular, genetic and clinical oncology in the past several decades the problem of lung cancer continues to plague medicine. Since the 1950s the incidence of lung cancer worldwide has been increasing and has reached now the proportion of an epidemic that has not yet leveled-off (1-3). Lung cancer kills yearly more than 170, 000 patients in the USA alone (2) and over a million around the world (3). In the United States, these deaths are more than those attributable to colon, prostate and breast cancer combined. The sheer scale of this epidemic has heightened efforts to understand the biology and molecular pathogenesis of lung cancer including lung cancer-causing genes and their interaction with environmental factors involved in cancer causation such as tobacco smoke, air pollutants and asbestos.

In this chapter we examine the basic facts and concepts in lung cancer pathogenesis. We will cover the following topics: (1) genetic and gene models in lung cancer; (2) cell models in lung cancer development including (a) lung embryology and stem/precursor cells related to lung tumors, (b) molecular evolution of initiated cells and field cancerization in the affected lung, (c) cellular evolution of the metaplasia-dysplasia-carcinoma in situ-invasive cancer sequence of events in the affected lung, and (d) molecular events in the obligatory sequence of progression to full blown invasive cancer in the lung; (3) classification, diagnosis, and clinical course of lung cancers; (4) treatment of all forms of lung cancers including (a) traditional combined regimens; (b) novel molecular targets for drugs discovery; (c) immunotherapy protocols; (d) gene therapy and gene delivery to the lung; (5) environmental factors as causative agents in lung

cancer pathogenesis and prevention of lung cancer; (6) optimism and pessimism for the immediate future in the post-genome era from the viewpoint of researchers, physicians, and patients.

1. Genetic and gene models in lung cancer causation

Epidemiological studies of human cancer(s) have long established (since 1940-1950) that carcinogenesis could be best approximated as a multistep process caused by genetic changes that accumulate over time (4, 5). As many as 6 to 7 such changes were considered necessary to bring about a full-blown malignancy that resulted in the patient's death. Later (in the 1960s), cell hybridization studies have established the recessive nature of the malignant phenotype (6). These earliest findings followed by monochromosome transfer experiments (7) held the promise of restoring the normal phenotype to cancer cells and sow the seeds of the "brave new world" of cancer gene therapy of today (8). Statistical analysis of familial and sporadic childhood retinoblastoma (RB) and Wilms' tumor (WT) have led Knudson to formulate the now classical two-hit hypothesis which postulates that two mutations are required and rate-limiting in the process of tumorigenesis (9, 10). In molecular terms, these double hits have been attributed to the inactivation of both alleles of a gene(s) serving a tumor suppressor function (hence, tumor suppressor gene(s), TSG) (9-11). Initially, there was not much progress probably due to the technological limitations at the time. Later the theory became amenable to a great range of applications and prompted an explosion of results. Combined research efforts correlated genetic predisposition to cancer with

specific abnormalities leading to the identification of a large number of oncogenes and TSG(s) (12). With the genes in hand, the predictions of the theory were verified and the mutated cancer-causing genes, oncogenes and TSGs, became the paradigm of genetic etiology and pathogenesis of human inherited and common cancers. The changing terminology in the field reflects the shift in the paradigm: from genes associated with cancer to, finally, genes causing cancer. To summarize, inherited (12), initiating (13), "gate keepers" (14), cancer-causing (our term) genes follow the two-hit proposition for cancer causation. These genes (TSGs or oncogenes) are usually discovered in the inherited family cancer syndromes through genetic mapping and positional cloning or alternatively through somatic deletion mapping in tumors and/or established cancer cell lines. Consistent with the two-hit proposition, the corresponding tumors show loss or inactivating mutations in the wild type allele (in family syndromes) (12, 15) or both alleles in sporadic tumors (in case of TSG) (15). In case of inherited activated oncogenes (16) the tumors may show loss of the wild type allele with simultaneous duplication/amplification of the mutant chromosome, or a second activating hit in the wild type allele (17, 18); same events happen in corresponding sporadic tumors (15). The net result of these rate-limiting genetic changes is a homozygous status for these cancer-causing genes in tumors. The recently discovered haploinsufficient TSGs (19, 20) apparently violate this rule. They predispose to cancer in the hemizygous state but do not require a second genetic hit in the tumor. Apparently, the second hit in this model may target another cancer haploinsufficient gene. Both cancer gene models apply to sporadic malignancies arising from the same stem cell(s) (see below, pp. xx).

A logical extension of these models assumes that multistage carcinogenesis would correlate with the stepwise most likely ordered inactivation/loss of several (TSGs) and/or with simultaneous activation of oncogenes (altogether not exceeding 6-7 events). While the activation/inactivation of the first cancer-causing gene in a normal stem cell would initiate malignant transformation, the subsequent changes in these initiated/transformed cells would drive clonal expansion, development, and progression into a full-blown tumor. The products of cancer-causing genes (oncogenes and TSGs) are components of signal transduction pathways and signal integration mechanisms that control cellular proliferation, differentiation, cell migration and/or apoptotic death. Identification of the components of these signaling pathways will help to understand cancer pathogenesis and lead to development of mechanism-based therapies (see below, pp. xx). It is important to stress that TSG transfer studies have established that a single normal individual gene can reverse the malignant phenotype despite the presence of other genetic defects (21, 22), pointing at molecular marks that would later be targeted with new selective drugs and other treatment modalities (23).

We now enumerate and describe briefly some of the most important cancer genes (oncogenes, TSGs, growth related genes, death related genes) involved in the origin and/or development of lung cancer(s) and then provide a tentative gene model of lung cancer pathogenesis. Over a 100 oncogenes and about 50 TSGs have now been described (15, 24) approaching the expected number of cancer genes (i.e. 200) as suggested by the number of distinct human tumors (25). A comprehensive search of the human genome draft sequence for paralogous sequences of known cancer genes did not result in finding such genes (26, 27).

Oncogenes

The RAS genes play a fundamental role in signal transduction pathways involved in cellular proliferation and apoptosis interacting with other regulatory circuits of cell growth and death (23, 28). Activation of the K-ras proto-oncogene by point mutations in codon 12 accounts for over 90% of RAS mutations in lung cancer. It occurs in 50% of lung adenocarcinomas and in 20% of all NSCLCs but not at all in SCLCs or other lung tumors with neuroendocrine features or squamous cell carcinomas. The K-ras codon 12 point mutations are found by PCR in bronchoalveolar cell lavage and predict poor prognosis of these patients. The recently discovered RASSF1 TSG family (see below, pp.xx) from the 3p21.3 lung and breast cancer region (22, 29-31) encodes proteins with a RAS binding domain. We propose that the RASSF1 products sequester RAS proteins and therefore negatively regulate their concentration in the cell. In this model, over-expression of RAS or inactivation/loss of RASSF1would liberate RAS and drive proliferation; similarly, the same endpoint would be obtained if mutated RAS protein would not bind to RASSF1 protein. Consistently, the RASSF1 locus is silenced in about 100% of SCLCs and only in about 30-50% of NSCLCs (30, 31) correlating with the frequency and distribution of RAS mutations.

The MYC genes comprise a family of related genes (c-myc, N-myc, L-myc) that encode transcription factors of the BHLHB2 type. The product of c-myc forms a heterodimer with Max and activates genes involved in growth control and apoptosis (32). The most common abnormality involving the Myc genes in lung cancer is gene amplification or

gene over-expression: over-expression with amplification reached 80-90% in SCLCs (23 but only 50% in NSCLC specimen without gene amplification (23).

The BCL-2 gene product is a key member of the normal pathway of programmed cell death and when over-expressed protects cell from apoptosis (23). It is highly expressed in SCLCs (33) and much less frequently in NSCLCs (34).

The ERB-2 gene encodes a receptor tyrosine kinase and due to amplification is over-expressed in 25% of NSCLCs (23, 28) and rarely expressed in SCLCs (23, 28). It is a biomarker of poor prognosis in NSCLCs (23) and a target for therapy with specific antybodies (23). The related epidermal growth factor receptor, **ERB-1**, is activated in lung cancer cells by over-expression (23).

The MDM-2 gene encodes an oncoprotein that binds to p53 and inhibits its transcriptional activity (35). It also targets wild type p53 for ubiquitin-mediated degradation (35, 36). It is rarely amplified in NSCLCs (37), however its product is observed more frequently by immunohistochemistry and correlates with better prognosis among patients without p53 accumulation (38).

The MST1R/RON gene encodes a receptor tyrosine kinase (RTK) and together with Met (see below) comprise a unique two-member RTK family involved in lung cancer. RON is highly expressed in normal lung (39, 40), in SCLCs a truncated form of the mRNA is universally expressed while in NSCLCs only the long apparently normal mRNA is present (40). In lung cancer activating mutations in the tyrosine kinase domain were extensively searched for but none were found yet (40). Recently, we discovered that the RON/MST1R locus encodes also for a truncated protein that has a constitutively activated tyrosine kinase domain and is encoded by the short mRNA in SCLCs (Angeloni

et al, unpublished); similarly other investigators discovered a short form of Met (41) (see below). These two RTKs form a unique two-member family and have an identical modular and domain structure and therefore perform identical functions. We propose the following model involving this family in developmental and adult growth and their deregulation in tumor growth. In normal growth, the long form, RTK, and the short form, TK, are never expressed simultaneously in the same cell; when the promoter of the long, RTK form is silenced by de novo methylation (Angeloni et al, unpublished) the short form, TK, is expressed by default. In this model, the TK form drives proliferation to expand a specific cell population to a certain controlled number; then the RTK promoter becomes activated again (by demethylation) and the RTK activated by specific ligand binding drives differentiation of the expanded cell compartment. The net result is normal growth. When coupled with programmed cell death this process would account for homeostasis of adult tissues and organs. In SCLCs the RON RTK form is silenced and the TK form highly expressed and becomes constitutively activated and oncogenic; the mechanism of RTK activation in NSCLCs remains at present unknown.

The Met gene encodes a similar RTK and is involved in a number of human cancers (42) including hereditary and sporadic papillary renal carcinomas as a cancer-causing gene (17,18). The gene is expressed in normal lung (43) together with its ligand, HGF (43). This pair is also expressed in NSCLCs and high levels of HGF were associated with poor prognosis (44).

Death related genes

The FAS gene product and its ligand, FASL activate cell death pathway(s) in many cell systems (23). In the immune T-cells binding of FASL to the cognate receptor FAS, activates apoptosis and serves as a negative regulator of the immune response to tumor cells. Tumors employ a variety of strategies to avoid surveillance and destruction by the immune system (23). The FASL product is expressed universally, in all SCLC/NSCLC cell lines and in 80% of SCLC and 93% of NSCLC tumors mediating death of the immune T-cells (23). Since as a rule the tumor cells do not express the FAS receptor they avoid autocrine-induced FAS-mediated death (23). Recently, the candidate TSG locus on 3p21.3, LUCA-15 (29), was shown to suppress FAS-mediated apoptosis in Jurkat cells (45) by up-regulating the expression of the apoptosis inhibitor, BCL-2 (23), further implying a role in tumor growth.

Growth factors

Several growth factors (such as gastrin-releasing peptide, substance P, bombesin, EGF, TGF alpha and beta and others) and their cognate receptors show dysregulated expression in lung cancer creating a network of autocrine and paracrine pathways (23,46) that results in uncontrolled proliferation. Recently these pathways were targeted for treatment by antibodies (23), and antisense technologies (23,46). Recently the insulin-like growth factors, **IGF-1 and IGF-2** and their receptors were shown to be important in the development of all forms of lung cancer (23) prompting clinical trials with somatostatin to block the IGF-IGF-receptor complex function (23).

Tumor suppressor genes in lung cancer

The paramount importance of tumor suppressor gene (TSG) inactivation in human cancer causation was stressed and briefly discussed above along with cancer gene models (see pp. xx). Much progress was achieved earlier in the identification of TSGs involved in lung cancer development and /or progression but only recently novel TSGs residing in the chromosome 3p21.3 region were identified that represent bona fide lung cancer-causing genes (22, 29-31, 47).

Two critical cell cycle regulatory pathways, namely the RB (23, 48) and p53 (23, 35) TSG pathways are deregulated in all types of lung cancer consistent with the general view of cancer as a "disease of the cell cycle" (23, 35, 48).

The RB pathway controls the G1 checkpoint and allows the transition into the G2/S-phase of the cell cycle (23, 48). Incoming growth-promoting signals transduced from cell-surface receptors to the nuclei cause rapid and transient elevation in D-cyclins in early G1-phase. In particular, cyclin D1 activates Cdk4/6 kinase that phosphorylates the RB protein (pRB) resulting in the release of the transcription factor E2F which in turn activates S-phase genes including thymidine kinase, Cdc6, c-Myc, and DNA polymerase-alpha (48). The RB pathway also includes the p16/MTS1 (multiple tumor suppressor gene from 9p21) which serves a Cdk inhibitor function for the D-Cdk4/6 dimers, causing G1 arrest. Deregulated passage trough the G1 checkpoint into S-phase would result either from inactivation/loss of one of the tumor suppressors (p16 or pRB) or activation of cyclin D1 by mutations or over-expression (48). Loss of RB or p16 or cyclin D1 over-expression occurs in most, if not all, human cancers including lung cancers, and constitutes a necessary step in cancer development and /or progression (48). In addition, the E2F gene is frequently over-expressed in lung cancer (23). Clearly, RB is a cancer-

causing gene for retinoblastoma and osteosarcoma (49) while p16 causes familial melanoma (50).

The p53 pathway. The p53 TSG is the most frequently mutated gene in cancer (35) and is mutated in 50-60% of NSCLCs (23, 35) and 70-90% of SCLCs (23, 35). Mutations in p53 commonly reflect exposures to environmental carcinogenes, e.g. cigarette smoke in lung cancer. The p53 protein has been frequently referred to as the "guardian of the genome" (35) because the p53 gene is induced by DNA damage that leads to cell cycle arrest at the G1/S border until DNA is repaired. If repair is delayed, it steers the damaged cells into apoptosis (35). Cell cycle arrest is achieved by p53 inducing the expression of p21, a Cdk inhibitor. Alternatively, apoptosis is induced by bax whose expression is again induced by p53 (35). Loss of p53 therefore results in deregulated cell cycle progression and accumulation of DNA damage i.e. mutations or genomic instability. The Li-Fraumeni cancer family syndrome is caused by germ line mutations in the p53 TSG (49) and manifest multiple types of cancer that do not include lung cancer (50); however surviving affected patients may develop multiple primary lung cancers of different types (51). They usually occur late and are associated with heavy smoking suggesting that p53 is not a cancer-causing gene in lung cancer.

The RASSF1 genes from 3p21.3. Loss of genetic material from chromosome 3p21.3 by heterozygous or homozygous deletions is the earliest, most likely the first, genetic event observed in many types of lung pre-malignant lesions. It was also observed in histologically normal-looking, tobacco smoke-exposed, matching bronchial epithelium (52), indicating the presence of activated lung cancer-causing genes. Recently, 3p21.3

was extensively analyzed and all resident genes were cloned and characterized (29). At present, the resident RASSF1 genes, RASSF1/A and RASSF1/C, driven from separate CpG-type promoters and sharing four terminal exons, were shown to represent bona fide TSGs (22, 29-31, 47). The corresponding proteins share a common RAS binding domain; in addition, RASSF1/A contains a diacylglycerol (DAG) and SH2 domains in the amino-terminal half of the protein. The presence of these domains suggest the RASSF1 protein could play fundamental role(s) in signal transduction pathways from the cell surface to the nucleus. We hypothesize that the RASSF1 protein binds RAS and thereof controls the amount of RAS available for signal transduction. Therefore, inactivation or loss of RASSF1 would result in "over-expression" of RAS, which would in turn, drive malignant proliferation. RASSF1/A is silenced by promoter hypermethylation in over 90% of SCLCs and SCCs and in about 50% NSCLCs (correlating with the mutational status of RAS) and is able to suppress growth of lung cancer cells in culture and tumor formation in mice (22, 29-31). Moreover, the gene is silenced in many human cancers including kidney (22), breast (31), head and neck (53) and prostate (Kuzmin et al, unpublished, 2001), probably due to its ability to control RAS. It is therefore tempting to assume that RASSF1/A is a multiple tumor suppressor gene and is probably involved in development or progression of a majority of human tumors. However in lung cancer it is rather a gatekeeper cancer-causing TSG because its inactivation/silencing is probably the first abnormality in the sequence of events leading to pre-malignant lesions and then invasive cancer (see below, pp. xx). The RASSF1/C gene is involved in the development and/or progression of ovarian cancer (54).

The HYAL2/LUCA2 gene from 3p21.3 region. Recently, this candidate TSG that resides in the 120 kb critical 3p21.3 segment (29) has been identified as a cell-surface GPI-anchored receptor for jaagsiekte sheep retrovirus (JSRV) cell entry (47). JSRV is an ovine retrovirus. In sheep, it causes a contagious form of lung cancer that arises from epithelial cells in the lower airway including type II alveolar and bronchiolar epithelial cells (55). The sheep tumors exhibit histological features similar to many human pulmonary adenocarcinomas, including bronchiolo-alveolar carcinoma (BAC), whose incidence is rising and has now replaced the incidence of SCC (56). It was also shown that the En*v* gene of JSRV could induce foci formation in rodent cultured fibroblasts (47) and human immortalized bronchial epithelial cells (Danilkovitch et al, unpublished, 2001), thus identifying Env as the oncogenic factor in lung cancer pathogenesis. The pathogenesis of BAC is quite peculiar and could be explained by infection with a human retrovirus similar to JSRV, therefore suggesting the possibility of viral etiology of BAC. It appears that JSRV does not cause lung cancer in humans having occupational exposure to this virus (55). However, a recent study showed that antiserum directed against the JSRV capside protein cross-reacted with 30% of human pulmonary adenocarcinoma samples but not with normal lung tissue, or many adenocarcinoma samples from other tissues (57), supporting the proposition that related viruses may indeed be involved in human lung cancer. The Env of this hypothetical virus could sequester the product of the HYAL2/LUCA2 TSG and thus liberate an oncogenic factor negatively controlled by HYAL2/LUCA2 TSG. This factor, together with the Env protein, would drive malignant transformation of bronchial-alveolar epithelial cells. Experiments with JSRV transformed human bronchial epithelial cells (Danilkovitch et al, unpublished, 2001) provide support

for this model. The viral etiology for BAC has a precedent in viral etiology of several human cancers that include cervical cancer and HPV (58) and mesothelioma and SV40 (59). Importantly, both SV40 and HPV proteins sequester TSG products, p53 and pRB respectively (35, 48) inactivating the respective pathways.

Lung cancer gene models

Large and punctuated allelic losses in the chromosome 3p21.3 region are present in perhaps all types of lung cancer (29, 52), their pre-malignant precursor lesions (52) and even in the histologically normal bronchial epithelium of current and former smokers (29, 52). This indicates that TSG(s) residing in such chromosomal region are likely to play a causative role in the earliest steps of lung cancer pathogenesis. Chromosome-transfer studies demonstrating the presence of a tumor-phenotype suppressing function in 3p21.3, lends further support to this proposition (60). Increasing evidence suggests that more than one gene in the 3p21.3 region (which contains at least 8 critical candidate TSGs - see above, pp.xx and ref. 29) can serve as TSG in lung cancer causation. Among these genes some conform to the classical two-hit model requiring inactivation/or loss/or silencing of both alleles of the gene (homozygous, -/-, phenotype), while others may function as haploinsufficient TSGs one allele of which is still expressed in the tumor (hemyzygous, -/+ phenotype). Since the acquisition of other genetic modifications is still rate-limiting in causing cancer (15), another mutation in a different gene would appear to be required in the haploinsufficient cancer model. It is tempting to assume that it could be a mutation in a second known TSG such as p16, *p53*, and *RB*, all of which are frequently mutated in many common cancers bearing 3p21.3 allele loss, including lung cancers (29).

Consistently, allele loss at these genetic loci (at 9p21, 13q14 and 17p13) occurs after 3p allele loss at a later stage of carcinogenesis when histologically dysplastic or carcinoma *in situ* lesions become evident (see below, pp .xx).

Lung embryology (see below, pp.xx) identifies lung stem cells as the primitive columnar epithelial cells derived from the primordial upper gut at days 40-45 of development (61). These columnar cells first differentiate into neuroendocrine cells (probably, the cells of origin of SCLCs), and a variety of multipotent bronchial epithelia cells (probable the cells of origin of NSCLCs). In fact some lung cancers, exhibit within the same tumor histologic features of several lung cancer types indicating a possible common stem cell of origin (29). The embryology of the lung would therefore suggest that the same genes could be involved in both SCLC and NSCLC carcinogenesis.

The following model of lung cancer pathogenesis based on these developing concepts and accumulated evidence is proposed. Consequent to smoking (or other environmental pollutants) damage, 3p21.3 allele loss occurs in thousands of different sites throughout the respiratory epithelium leaving the putative 3p21.3 TSGs haploinsufficient. These initiated (3p21.3 hemizygous) cells proliferate and spread throughout the lung epithelium and/or form clonal patches estimated to consist of ~50-100,000 cells (~15-17 doublings). The next hit could occur either in the second allele of a classical TSG (e.g. RASSF1) or, as required in the haploinsufficient model, in another cancer-causing gene (such as *RB*, *p53*, p16 or "*gene X*"), leading to the next stages of invasive cancer. The *RB* and *p53* genes are mutated in nearly 100% of SCLC samples, while mutations of *p53* occur in 50% of NSCLC and some form of *p16* inactivation (also inactivating the RB pathway) occurs in a very large fraction of NSCLCs (23). In the classical homozygous model the

3p21.3 TSG first undergoes allele loss and then a second inactivating event (either as uncommon mutation or loss of expression through promoter hypermethylation) which is required to allow the clonal outgrowth of these initiated cells. At any rate, the nearly universal loss of 3p21.3 DNA in SCLC and squamous cell carcinoma of the lung (SCC), and its occurrence in over 50% of adenocarcinomas of the lung, suggest that these deletions are obligatory, rate-limiting steps in the pathogenesis of many lung cancers, if not all. It should also be noted that allele loss that includes 3p21.3 and the immediately surrounding 3p21 regions would lead to a condition of hemyzygosity for many other predisposing genes residing in the area. These include *MLH1,* TGFRII, beta-catenin, RON, and Wnt5, which also could contribute to malignant transformation. Functional testing by gene transfer into lung cancer cells (29-31) and gene disruption strategies in mice are necessary to test the theoretical model and discover all putative TSGs in 3p21.3, and also to produce mouse models of lung cancer. Such experiments are now in progress. Studies of familial lung cancer risk, including data from lung cancer occurring in young non-smoking individuals, are compatible with Mendelian codominant inheritance of a rare major autosomal gene that produces earlier age of onset of lung and probably other cancers (29). In due time a classical TSG(s) (with homozygous inactivation in tumors) segregating in these rare lung cancer pedigrees, could also be discovered. In any event, the early involvement of chromosome 3p21.3 allele loss in pre-malignant lesions and sporadic lung cancers argues that one or more of these genes play a causative role in the origin and/or development of common, sporadic lung tumors.

2. Cellular models in lung cancer development

Lung embryology and stem/precursor cells related to lung tumors.

From an evolutionarily perspective, lungs are a relative novelty that appeared about 400 million years ago with the purpose of supplying oxygen to the heart tissues of evolving Vertebrates (for a review see 62). Lung development extends from branching morphogenesis in early embryonic life, through the critical transition from fetal life to air breathing up to the postnatal completion of alveolarization.

With about 17 million branches and 70 m^2 of epithelial gas-exchange surface (63), human lungs can support oxygen consumption ranging between 250 ml/min at rest and 5500 ml/min during hard exercise. Simultaneously the matching capillary network can accommodate a blood flow rising from 4 l/min to 40 l/min in the transition from rest to maximal exercise (63).

The lungs derive from the digestive tube (64). Their origin can be traced back to a ventral outgrowth (laryngotracheal groove) that develops from the pharyngeal floor, between the fourth and sixth pharyngeal pouches (Fig. 1) The groove deepens and grows downwards to form a pouch-like evagination, fully open to the foregut. On either side of the groove, two longitudinal folds of tissue (tracheo-esophogeal folds) grow together and fuse forming a new tube (laryngeo-tracheal tube) distinct from the fore-gut. Communication with the foregut is maintained via the longitudinally oriented laryngeal orifice.

Proliferation of the underlying mesenchyme forms swellings around the laryngeal orifice from which the epiglottis, glottis, laryngeal cartilages and musculature will develop. At the same time, the laryngeo-tracheal tube elongates downwards and penetrates the underlying mesoderm. A distinct swelling develops at the distal end and is termed the lung bud (respiratory diverticulum). The laryngo-tracheal endoderm constitutes the lining of the trachea, bronchi and lung alveoli. From a histological point of view, human lung development proceeds through five partially overlapping phases (64).

Embryonic phase (3-7 weeks). Approximately 28 days after fertilization, the lung bud branches from the primitive fore gut to form the left and right primary bronchial buds, which will ultimately develop into the left and right lungs. Interaction of the epithelium with the underlying splanchnic mesoderm controls the branching events. By the fifth week, elongation, branching and budding of the two bronchial buds give rise to three bronchial stems on the right and two on the left. The formation of the presumptive bronco-pulmonary segments provides the foundation for the lobular organization of the mature lungs and ends the embryonic phase.

Pseudo-glandular phase (7-16 weeks). About 21 further orders of branching of the duct system generate the presumptive conducting portion of the respiratory system, up to the level of the terminal bronchioles. At this time, the airway lumina, narrows and lined with pseudo-stratified squamous epithelium, are embedded within a rapidly proliferating mesenchyme. The structure has a glandular appearance. From week 13 onward, the lumina enlarge and the epithelium thins to a more columnar structure. The pluripotent epithelial cells differentiate to ciliated cells and goblet cells, progressing from the proximal to the distal regions of the developing lung.

Canalicular phase (16-24 weeks). The terminal bronchioles divide to form two respiratory bronchioles. This time is also characterized by extensive angiogenesis to form a dense capillary network within the rapidly proliferating mesenchyme that surrounds the more distal tract of the embryonic respiratory system. Dichotomous branching continues. The cuboidal intermediate cells of the lower airways differentiate to form ciliated cells and Clara cells. Differentiation of the mesenchyme begins during week 10 and progresses along the developing respiratory tree giving rise to chondrocytes, stromal fibroblasts and myoblasts. Differentiation of the mesenchyme and epithelia begins in the more proximal regions of the airways and progresses distally. The pulmonary arteries and veins develop in parallel with the conducting portion of the lungs.

Terminal sac phase (24-36 weeks). The primitive alveolar ducts continue branching. The terminal sacs grow. Continued thinning of the stroma brings the capillaries into apposition with the prospective alveoli. The cuboidal cells of the terminal sac epithelium differentiate, via several intermediate stages, into alveolar type II cells, that secrete low levels of surfactants. Type I pneumonocytes differentiate from cells with a type II like phenotype. Type I cells then flatten, increasing the epithelial surface area by dilation of the saccules, giving rise to immature alveoli. By week 26, a rudimentary though functional blood/air barrier has formed. It is still insufficient, in case of pre-term born infants. Maturation of the alveoli continues by further enlargement of the terminal sacs, deposition of elastin foci and development of vascularized septa around these foci.

Alveolar phase (36 weeks - term/adult). Fully mature alveoli begin to appear around week 36. They are characterized by thin walled inter-alveolar septa with a single layered capillary network. The diameter of the capillaries is sufficiently large that they may span

the alveolar walls and interact with the air spaces on both sides. New alveoli continue to form at high rate after birth for up to 3 years. Thereafter, all components grow proportionately until adulthood.

Histological typing of the airways epithelia. Different epithelial cell lineages are arranged in the airways following a proximo-distal spatial pattern. The larynx is lined with squamous epithelium and the upper airways are lined with ciliated columnar cells, basal cells and mucous secreting cells. The apical end of the ciliated cells shows abundant cilia (200 or more) projecting into the airways lumen. Because of their inability to incorporate [^{3}H]-thymidine, ciliated cells are supposed to be differentiated cells incapable of further division, although this is still a controversial issue (65).
Basal cells are small cells that contact the basal membrane of the proximal airways, but do not extend up to the airway lumen. Adult lung basal cells are able to incorporate [^{3}H]-thymidine, indicating that they normally proliferate. However, it is currently hypothesized that these are not the stem cells of the lung epithelium (66), although they may play a role in maintaining the lung epithelium architecture by forming desmosomes with columnar cells and hemidesmosomes with the basal membrane. The lower airways are lined with Clara cells. Clara cells are non-ciliated secretory cells that can be localized throughout the respiratory tree, but in the lower airways they have been shown to be the most actively dividing cell type during pre- and post-natal development (66). Clara cells have been attributed a number of critical roles such as being progenitors for the ciliated cells as well as for new Clara cells (67).

The alveoli are lined with alveolar type I and type II epithelial cells. Type I pneumocytes are recognized by flattened surface, protruding nucleus and complex cytoplasmic processes. They represent 33% of the total cells but cover about 97% of the total surface. Type I cells generate a tight sheet of cells covering the alveolar septum to form the pulmonary capillaries by fusing to the basal lamina of vascular endothelial cells. This structure forms the gas exchange barrier that is 0.2-0.5 μm thick. Type I do not have the capacity to divide. Type 2 are cuboidal in shape, show abundant mitochondria, endoplasmic reticulum, polyribosomes and a developed Golgi apparatus. They represent 67% of the total epithelial cells but cover only 3% of the surface area (68). Type II cells secrete surfactant proteins that are responsible of reducing the surface tension in the alveolar sacs. Type II cells are hypothesized to give rise to both type II and I upon injury of the distal epithelium (69). Type I can also differentiate into type II cells (69). This suggests the level of positive and negative/signaling going on to maintain a balanced epithelial structure and function. A level of even higher complexity can be inferred from injury studies based on the usage of different chemicals. Studies based on NO_2 have shown that Clara cells can serve as stem cells in the proximal epithelium and type II in the alveolar epithelium. In turn, studies based on naphtalene, that selectively targets and destroys Clara cells, showed that ciliated cells in the proximal airways and PNE in the terminal ends – that lack ciliated cells – are responsible for repopulating the injured lung epithelium (70). Pulmonary neuro-endocrine (PNE) cells are situated in small foci called neuroepithelial bodies and are surrounded by the other epithelial cells in the upper airways. PNE cells are among the first cells to differentiate from the primitive lung epithelium. PNE cells are surrounded by a greater density of proliferating cells than

elsewhere in the epithelium, and express a number of proliferative cytokines, including calcitonin-like peptide and bombesin. Bombesin-like peptides (BLP) promote branching morphogenesis and stimulate epithelial and mesenchymal cell proliferation, type II differentiation, surfactant phospholipid synthesis, and secretion, as well as Clara cell and PNE cell differentiation (71). It has been hypothesized that, through BLP and calcitonin-related peptides release, PNE cells work in lung development stimulating lipofibroblast in the lung mesenchyme to interact with the airway epithelium, thereby regulating type II differentiation (72). PNE cells maintain a limited capacity to proliferate (73).

Lung stem cells. The many specialized cell types that make-up the lung epithelium have lineage relationships that are not fully understood. Therefore the mechanisms by which the pulmonary epithelial cells proliferate and terminally differentiate require much investigation. Besides the lung epithelium continually interacts with environmental damaging factors requiring constant tissue turnover or repair that are driven by lung-specific stem cells. Therefore identification of adult lung stem cells would help understand the natural history of all types of lung cancer.

Molecular evolution of initiated cells and "field cancerization" in the affected lung.

Tumors originate by clonal expansion of a single cell that has accumulated several genetic changes that provide a growth advantage over the neighboring normal cells. It is now accepted though that epithelial tumors rarely consists of a single clone, but rather more often of a mixture of separate clones that also had acquired the characteristic of genetic instability (74, 75). This genetic instability plays a central role in the Darwinian

evolution of expanding clones of initiated cells during tumor development and growth (76). The problem of clonality in lung cancer and the evolution of “initiated” cells to fully malignant cells and tumors is also associated with the “field cancerization” model of carcinogenesis in epithelial sheets (77).

Consequent to smoking damage, genetic changes occur in thousands of different sites throughout the respiratory epithelium creating clones of initiated cells. These clones undergo a phase of Darwinian evolution that selects those more capable of circumventing environmental problems such as hypoxia, malnutrition, immune system attacks, etc. Those clones that emerge after this stage carry on the invasive step. Each patch therefore may evolve into a distinct clonal tumor; close patches may fuse and develop further into polyclonal tumor and so on. From a clinical perspective, finding synchronous primary tumors is not unusual. These tumors frequently exhibit dissimilar hystology and distinct genetic signatures (78).

Cellular evolution of the metaplasia-dysplasia-carcinoma in situ-invasive cancer sequence of events in the affected lung.

What are the molecular events that cause the progression of an initiated cell to invasive cancer? We must distinguish between SCLC and NSCLC. In fact, although our knowledge of the events accompanying the rise of SCLC is quite inadequate, it seems that these tumors may arise directly either from normal or mildly abnormal epithelia. In NSCLC, instead, several premalignant stages have been described.

Small cell lung cancer (SCLC). SCLC is predominantly a central tumor. No specific preneoplastic changes have been described for SCLC, but several smoking-related changes such as squamous dysplasia and carcinoma in situ have been found associated with these tumors. The bronchial epithelium accompanying SCLC was found to show more extensive damage in terms of LOH than bronchial epithelium of patients affected with NSCLC (89). On the basis of frequent presence of genomic abnormalities in the morphologically normal mucosa surrounding the small cell tumor, it has been suggested that this tumor may arise de novo from the bronchial mucosa, without going through a stage of morphologically recognizable preinvasive lesions. Neuroendocrine cell hyperplasia has not yet been extensively studied at the molecular level.

Non-Small cell lung cancer (NSCLC). In contrast with what happens in SCLC, NSCLCs appear to develop after a sequence of morphologic intermediate steps. As already mentioned, bronchial carcinogenesis is described as a multistep process. It involves transformation of the normal bronchial mucosa through a continuous spectrum of lesions consisting of basal cell hyperplasia, squamous metaplasia, dysplasia and carcinoma in situ (CIS) (79, 80, 81). In addition to these epithelial changes, alterations of the extracellular matrix (particularly of the epithelial basement membrane) are critical events in the development of invasion and metastases (82). The events leading from dysplasia/CIS to invasive disease are not completely understood but on the basis of animal experimental models (83), hyperplasia in the stem cell compartment of the epithelium seems to be the earliest response to environmental carcinogens. Exposure of the lung to environmental carcinogens induces changes in the epithelium, ranging from loss of cilia to basal cell hyperplasia (79). Genetic abnormalities accumulate before

histopathological changes are detected. As already discussed, genetic abnormalities most precociously found include 3p loss and 9p loss in metaplastic/hyperplastic lesions. Dysplastic lesions are characterized by progressive loss at 3p and, later, mutations of the p53 gene (84). Finally, 5q loss and mutations of K-RAS are found in late pre-invasive stages. Even if the first primary is removed, the remaining lung is still harboring multiple regions of clonal abnormalities. Continued carcinogen exposure constitutes a high risk for developing further malignancies.

Molecular changes caused by carcinogens can persist for many years after exposure cessation, in accordance with the long latency period often observed prior to the development of lung cancer (85).

The term “preinvasive” does not necessarily imply that progression to invasion would occur. In fact, some pre-invasive lesions are thought to regress after smoking cessation. However, other bronchial epithelial hyperplasia and metaplasia may occur that are not regarded as pre-neoplastic. These include goblet cell hyperplasia, basal cell hyperplasia, squamous metaplasia (86). Peripheral lung cancers, predominantly adenocarcinomas, may arise through a different mechanism and a different series of progenitor lesions (see below). The earliest and most common chromosomal aberration in broncogenic cancers is loss of DNA from the small arm of chromosome 3 (3p). In the mildly abnormal or even normal epithelium surrounding the lesions 3p loss is little and focal (87). In preneoplastic lesions the genomic loss is more pronounced (76% of hyperplasia and 86% of dysplasia are affected by 3p losses - 88). In CIS and invasive disease, at least five loci are lost (3p12-13, 3p14.2, 3p21, 3p21.3, 3p25). Also, losses at 9p21, 5q and 17p were described

(89). These changes occur early and are almost invariably present in dysplasia, CIS and invasive disease.

As expected from morphological studies, hyperproliferation seems to be an early event in bronchial epithelium transformation. The proliferative compartment was shown to rise from around 25% in normal epithelium to 35-40% in low and high grade dysplasias, to 85-90% in invasive squamous carcinomas (90).

p53 mutations, that follow the 3p loss, are also an early events in bronchial carcinogenesis (91). Telomerase deregulation appears to be part of the multistage carcinogenesis. Whereas most adult somatic cells have inactive telomerase, cancer cells at a point reactivate their telomerase activity possibly thereby preventing telomeres shortening and senescence. 70-80% of hyperplastic and dysplastic bronchial epithelium showed high telomerase activity (compared to 20% in normal controls, that raised up to 95-100% in CIS - 92). K-RAS is found mutated more commonly in adenocarcinoma than in squamous carcinoma. It appears lately in the development of some central bronchiogenic cancers arising through the hyperplasia-dysplasia-CIS sequence (93). Human papilloma virus (HPV) DNA has no importance in the genesis of bronchogenic squamous carcinoma (94) although, in some studies, HPV DNA was found in 18% of squamous carcinomas. Loss of p16 was found in moderate dysplasia and CIS, exclusively in lesions found in cancerous lung (95). Rb loss was seen in dysplasia/CIS but molecules in the Rb pathway (p16, Cyclin D1) were found abnormal in preinvasive squamous bronchial lesions. In particular, Cyclin D1 overexpression was found in early lesions (from 6% in hyperplasia to 38% of CIS lesions - 96).

Atypical adenomatous hyperplasia (AAH). It is now broadly believed that AAH is a preinvasive lesion, regarded as the adenoma in an adenoma-carcinoma sequence in the lung periphery. Immunostaining for p53 has been reported in 8-58% of lesions analyzed (97). LOH at 3p, 9p and 17p play a role also in the evolution of this lesion as they were found in 18%, 13% and 5% in AAH whereas the frequency raised to 67%, 50% and 17% respectively in the corresponding carcinomatous lesions (98). K-RAS mutations were found in 17-50% of AAH lesions (for a review see 99), suggesting that AAH represents an early step of adenocarcinoma, although clinical data in this sense are all but conclusive.

Molecular events in the obligatory sequence of progression to invasive cancer.

Several mutations involving tumor suppressor genes and oncogenes are required to allow the tumorigenic transformation of a cell and the rise of an invasive cancer. In lung cancer it has been shown that the neoplastic progression is not random but follows a pattern with genetic and genomic changes that are tumor-specific.

An elegant experiment (100) done in Barrett oesophagus gave a detailed map of the transformation events that happen in this type of epithelial tumor. The cytogenetic analisis of several biopsies taken during an extended period of time, showed that LOH at 17q and 9p, are found in premalignant epithelia several years before overt cancer. Mutations in p53 and p16 were also found before cancer, as well as hypermethylation of p16 promoter. Using specific p53 and p16 mutations, it was possible to follow the fate of different clones. It became evident that the process of transformation is somewhat more

complicated than a linear succession of events. In fact, cancer appears to be made of different clones originated from a common precursor but still evolving separately due to the acquisition of diverse mutations. Some clones disappear in time, whereas others become dominant in the cancer population. The general pattern of events comprises an initial step of clonal expansion of a given starting clone. The acquisition of new mutations projects the clonal population into a phase of genetic instability and evolution that goes on for years and ends with the production of pre-malignant cells. Noticeably, not all pre-malignant tissues progress to cancer.

3. Classification, diagnosis, and clinical course of lung cancers

Pathologists based on light-microscope observation of the malignant and surrounding normal tissue morphology first classified tumors of the lung. The WHO recently revised the histological typing of lung tumors and their pre-malignant precursor lesions, improving the earlier WHO classifications (101) by taking into consideration some recent advances in understanding lung tumors biology. An accurate classification of tumors is extremely important because it affects the decision on treatment to be administered. Clearly, a simple distinction on biopsy between SCLC and NSCLC is sufficient to predict the clinical course of the disease and sufficient to decide the treatment strategy. However, the morphological approach is fundamentally limited but, by necessity, still widely used (101). In the next several years, the rapid advance of genome research (especially now in the post-genome sequencing era) will provide with the ultimate discovery of all human genes, including cancer genes. This will be the foundation for a genetic classification of

cancers based on specific causative genes and cancer-gene expression profiling (102-104). The genetic approach will perhaps gradually replace the existing intrinsically limited morphological classification of human tumors. The new genetic typing of cancers will recognize the well-known phenomena of genetic and clinical heterogeneity of tumors. The genetic heterogeneity of cancers challenges our inability to distinguish tumors that appear similar in morphology but are caused by different genes. On the other hand, the phenomenon of clinical heterogeneity likely reflects the differences in morphology and topical expression of different mutated alleles of the same cancer-causing gene, may be coupled with expression of tissue-specific modifier genes and probably with the SNP make-up of individual patients. It is widely believed that genetic and expression profiling would better predict the clinical course of the disease and the design and outcome of treatment modalities (104, 105).

The better understanding of cellular models in lung cancer pathogenesis (see above) and the new histological typing of pre-malignant lesions (101), in conjunction with novel diagnostic tools for detection of pre-invasive cancer cells have provided good options for early detection of asymtomatic lung cancer. Combined use of novel physical methods (such as laser-induced fluorescence endoscope bronchoscopy (106) and low-dose spiral computed tomography (106, 107) with analyses for cytogenetic abnormalities in sputum and bronchoalveolar lavage cells, has shifted the clinical/therapeutic paradigm towards diagnosis and treatment of asymptomatic pre-invasive lesions to improve prognosis. In fact, the prognosis for patients with lung cancer strongly correlates with the stage of the disease at the time of presentation. Therefore, early identification and intervention strategies stemming from these developments are expected to improve the usually dismal

poor prognosis of this fatal disease. These multi-modality approaches to early detection, staging and therapy of specific types of lung cancers have undoubtedly improved the clinical course and outcome of the disease at least for early diagnosed types of lung cancers.

4. Progress in the treatment of lung cancers

Over time from the late 1950 until now, there has been slow but real progress in the treatment of lung cancers: the overall five-year survival rate has improved from 5% in the late 1950s to 14% in the middle 1990s (108). It continues to improve significantly due to advances in early diagnosis and combined modality therapies against both local non-invasive disease and more advanced forms of SCLCs and NSCLCs. These new multi-modality approaches include combinations of traditional treatments (surgery for NSCLCs, radiotherapy and multi-agents chemotherapy with cytotoxics and newer drugs, taxol and irinotecan, for SCLCs) with new drugs developed against defined molecular targets including signal transduction inhibitors, such as for example farnesyl transferase (109) and tyrosine kinase inhibitors (110), and drugs that attack tumor angiogenesis (111, 112). However, a word of caution is appropriate here since to much hype was introduced into the field. It is widely assumed and propagated in the post-genome literature that cancer cells may have or do have specific molecular targets whose discovery will be facilitated by the genome draft sequence. These are so called “druggable” targets for development of new specific drugs to affect (kill) cancer cells. However, most of such target molecules would be likely expressed in normal dividing cells of all renewable

tissues and certainly in tissue-specific normal stem cells, which obviously the wonder drug would also affect. The really specific molecular targets should belong to the category of molecules that are absolutely essential for cancer cell survival and proliferation and should not be expressed in normal dividing and stem cells. These are the molecules we need to discover to be able to approach the goal of cancer medicine, namely to cure cancer. Contrary to the existing beliefs we think that drugs against oncogene targets would attack normal dividing and stem cells and at the end of the day would be abandoned as well as oncogene antisense strategies. We have recently proposed that the cell surface trans-membrane carbonic anhydrases induced by hypoxia and highly expressed in tumor cells are excellent targets for drug discovery since normal dividing cells most likely do not express these enzymes (113,114). There are also indications that they might be altered by mutations in tumor cells. These cell proteins abundantly expressed on the surface of hypoxic cancer cells represent also excellent targets for immunotherapeutic strategies. Specific antibodies have been already developed and are being tested for treatment of kidney (115) and lung (116) cancers.

Naturally, the immune system (or should we say the “immunome” in keeping with the trend of creating “new” terminology) could be targeted against mutated proteins. It is widely assumed that it would find cancer cells expressing these mutated proteins operating inside the cancer cells (e.g. mutated RAS or p53 proteins) and would destroy them. This concept in our opinion does not hold water, nevertheless cDNA based vaccines are being produced against mutated cancer gene products that function inside tumor cells and are vital for their survival and growth. It is probably true that you can educate the immune system (the ‘immunome”) to recognize a mutated RAS protein as

non-self, but how the activated T cells would find their target inside live cancer cells remains a mystery. The usual explanation is that, like normal cells, cancer cells constantly process intracellular proteins to present them to the immune system, probably to commit suicide. Normal cells occasionally present their processed proteome to the immune system for surveillance purposes. This approach to immunotherapy of cancer probably originated also from the well-established facts that infected cells in a “fit-of-altruism” process the infectious agent and display it on their surface to activate the “immunome”. Since evolution and development of cancer cell populations is essentially a Darwinian process, it is inconceivable that cancer cells would do a similar thing and commit suicide. It is therefore obvious that the cell surface carbonic anhydrases would be a much better cancer target for production of cDNA-based vaccines. Needless to say such vaccines could also destroy normal highly differentiated cells that express these enzymes on their surface, however these cells would be replaced by the existing not affected stem cells. Recently, dendritic cells, potent professional antigen-presenting cells that can elicit primary immune responses to foreign antigens, became the focus of research directed toward their use in vaccine strategies for the treatment of cancer (117). These strategies use tumor-pulsed dendritic cells with tumor-associated antigens including whole tumor cell lysates (118). Undoubtedly in these experiments the tumor cell lysates contained processed carbonic anhydrase and probably other cell surface antigens.

The imperative of cancer research is to be able to prevent or cure cancer. The received wisdom holds that this ultimate goal of cancer medicine is in fact achievable. It is becoming increasingly clear that immunotherapeutic strategies as defined above and gene

delivery therapies (gene therapy) together with common sense prevention (see below, pp.xx) hold the keys to successful eradication of lung cancer.

Therefore, new horizons are being explored and substantial progress could be achieved in gene therapy strategies for lung cancer. Gene therapy approaches include: (a) replacing inactivated/lost causative TSGs with normal wild type alleles, (b) delivering genes that become toxic under controlled conditions (suicide gene therapy,); (c) delivering genes that interfere with autocrine and paracrine growth factors involved in lung tumor growth and (d) delivering genes that could augment the immune response and/or block tumor defenses (23, 119). The major impediment of gene therapy is the absence of ideal delivery systems (23, 119). Vectors range from viral (adenoviral and retroviral) to chemical, liposome, formulations and finally naked recombinant cDNA expressing the product (23, 119). However there is constant improvement in vector construction and formulation and there is hope that systemic delivery of therapeutic genes to primary and disseminated lung tumors will become a reality. In this regard the discovery that the candidate TSG HAYL2 is a receptor for the sheep lung cancer retrovirus, JSRV (47, 55), suggests that on the base of this virus, vectors for delivering genes to bronchial epithelial cells could be constructed.

We take the view that a combination of gene replacement strategies (delivering normal alleles of causative TSCs) with available immunotherapeutic approaches is a preferable course to combat lung cancer.

5. Environmental factors as causative agents in lung cancer pathogenesis and prevention of lung cancer

Lung cancer is a preventable malignancy since most if not all causative environmental factors that trigger the genetic changes leading to cancer were identified and extensively studied (120- 122). Here we focus on the modifiable risk factors such as tobacco smoking, diet, occupational exposure, and environmental pollutants that were identified as causative agents for lung cancer. We will also argue for early diagnosis and screening as preventive measures that should reduce the burden of lung cancer.

About 10-20% of smokers develop lung cancer during life and smoking causes >90% of all lung cancers (121). However since bronchioloalveolar adenocarcinomas contribute now ~25% to lung cancer and are not associated with smoking (123) only ~75% of lung cancers should be attributed to smoking. Tobacco consumption is also causally related to cancers of the colon, mouth, larynx, esophagus, bladder, kidney and pancreas increasing the burden of cancer death due to smoking to more than 30% in the United States. Common sense dictates that tobacco use should be avoided especially in young age since early onset of smoking is related to higher risk of lung and colon cancers. Therefore a broad range of social measures are now mobilized to succeed in preventing adolescent smoking and persuade long term adult smokers to stop smoking. Cessation of smoking usually reduced the risk of lung cancer after 5 years from cessation; the risk continues to decline further with duration of time (120-122). Benzo[a]pyrene the most active of the 20 carcinogen in tobacco smoke and tar was shown to rapidly (within minutes) attack and destroy promoters of actively transcribed genes followed by incomplete repair of some (124); this mechanism could lead to silencing cancer-causing TSGs and therefore initiate the malignant transformation in lung and other susceptible tissues. Footprint mutations

due to benzo[a]pyrene in other genes (such as p53) were shown (125). Individual variability in lung cancer risk and carcinogen metabolism, mainly via metabolic polymorphisms in the carcinogen metabolizing enzymes (126, 127) may explain differential susceptibility of smokers to lung cancer. In addition, some of the known occupational lung carcinogens, including asbestos, arsenic, coal gas, chromates, nickel, and silica (120) were shown to interact with smoking to increase the risk of lung cancer multiplicatively (i.e. effects proportional to the effect of smoking). However, it was shown in cohort studies that the relative risk of lung cancer from asbestos exposure is about twice as high in non-smokers compared to smokers and recently concluded that the multiplicative hypothesis is untenable (120, 128). This suggests that the effects of combinations of smoking with other environmental factors and carcinogens remain to be carefully studied. As of asbestos exposure the recently conducted meta-analysis study concluded “that besides mesothelioma, lung cancer is the only malignancy that demonstrates unequivocal association with asbestos exposure” (128, 129).

Recently an international group of scientists proposed an immediate ban on production and use of asbestos products (130). Other risk factors such as arsenic and industrial pollutants, radon and radiation exposure from occupational, medical and environmental sources, and diet and nutrition, collectively cause about 10-15% of lung cancers (121, 122) not directly related to smoking.

Clearly, we know now that most if not all lung cancers are caused by environmental factors that interact with the lung epithelial cells directly or after being modified by carcinogen-bioactivating (detoxifying) enzymes to introduce mutations into cancer-causing gene loci, TSGs and oncogenes. We also know that the current epidemic of lung

cancers continues unabated despite all efforts to limit contact with environmental risk factors. The legacy of decades of tobacco use in a sizable proportion of the current US population suggests we need to turn to strategies of early diagnosis and chemoprevention to reduce deaths and suffering of those destined to develop lung cancer. The potential of new screening procedures to detect even pre-malignant lesions in lung epithelium in high-risk populations have been recently demonstrated by the results from the Early Lung Cancer Action Project (131); these observations emphasize that lives could be saved by early detection and treatment of asymptomatic lung cancer patients (132). These findings also provide the rationale for development of chemoprevention strategies for patients with asymptomatic and localized disease (133). In principle, chemopreventive substances aim to prevent genetic damage inflicted by carcinogenic agents or aid in DNA and/or tissue repair to prevent mutations or eliminate initiated cells. Retinoids taken orally have shown recently promising results in preventing oral cancer (134) and reducing risk of second primary cancers in patients with head and neck cancer (134). However chemoprevention of lung cancer (135, 136) in large-scale clinical trials such as EUROSCAN (i.e. the European Study on Chemoprevention With Vitamin A and N-Acetylserine) met with little or no success at all (135-137). Similar negative results were obtain in other large-scale studies, namely, the ATBC (Alpha-Tocopherol, Beta Carotene) in Finland (136) and the CARET (Beta-Carotene and Retinol Efficacy Trial) in the United States (138) suggesting that prior carefully designed basic mechanistic studies are needed to attempt to approach chemoprevention of lung cancer. However a word of optimism is also needed in this difficult and already frustrating area of cancer research; an increasing number of agents should keep clinicians hopeful.

6. Hope and pessimism still rule in cancer medicine

Since the 1970s when President R. Nixon inaugurated the War on Cancer Act the United States spent enormous amount of monies and effort to discover the genetic causes of cancer and find a cure. At that time (the 1970s when one of us, MIL, was still young) there was a cartoon shown on the International Congress of Biochemistry depicting the cancer problem from different perspectives. It showed a researcher in a blue lab-coat saying he just made a discovery that would lead to a new powerful treatment, next to him was a skeptical physicians sitting on the patients bed and saying that "they" always come-up with things that wouldn't work. The desperate patient was asking for anything that would help him. Did this picture change?

Currently we know much about cancer causation but the magic bullet/cure is still elusive. However we take the view that for several reasons now is really the time for some optimism. Firstly, the soon to be completed (2005?) and understood (2020?) human genome sequence holds the promise that all cancer genes will be identified and their functions deciphered. Secondly, genetic individual differences that determine susceptibility to environmental risk factors and control the response to treatment modalities also would be identified along with ways to manipulate them. Thirdly, with the genes and their products in hand new modalities would be found to prevent and combat/cure cancer. This optimistic view has become the core belief of both researchers and clinicians facing the constant onslaught of cancer.