Surg Today (2007) 37:725–734
DOI 10.1007/s00595-007-3516-6

SURGERY TODAY

Review Article

Impact of Smoking Status on the Biological Behavior of Lung Cancer

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Abstract
Cigarette smoking is the most established risk factor for lung carcinogenesis; however, its effects on the progression of lung cancer are still unclear. We reviewed the clinical investigations on this issue, which imply that smoking status is a treatment predictor and prognostic factor for several subtypes of lung cancer. Moreover, gene alterations and various protein expressions of tumor progression were recognized more frequently in the tumor tissues of smokers than in those of the never smokers. A cellular analysis revealed that tobacco-specific chemical compounds cause genetic or epigenetic alterations, modulate expressions of large numbers of genes that include molecules related to proliferation, invasion and metastasis, and deteriorate anti-tumor immunity. Our findings suggest that smoking promotes the progression of lung cancer, and that elucidating the molecular mechanisms may help to clarify the therapeutic targets.

Key words Lung cancer · Tobacco smoking · Loss of heterozygosity · Methylation · FHIT · Hexokinase · Hypoxia-inducible factor · Benzo(a)pyrene · Polycyclic aromatic hydrocarbon

Introduction

Tobacco-related deaths now exceed 5 000 000 each year worldwide. Lung cancer, one of the major tobacco-related diseases, is a leading cause of cancer death in the world, and its incidence is still increasing. Tobacco smoke-derived toxic compounds such as aromatic hydrocarbons have been proved to cause lung carcinogenesis,[1,2] and the increasing mortality from lung cancer is closely associated with the consumption of tobacco.[3] Despite campaigns to prevent people from smoking in an attempt to decrease lung cancer mortality,[4] the population of smokers worldwide continues to increase.[5,6] It remains unclear whether tobacco smoking affects the progression of lung cancer. Benzo(a)pyrene [B(a)P], one of the major carcinogens in cigarette smoke, can induce molecules that cause inflammation, and cellular proliferation and migration. Thus, it has been suggested that tobacco smoke-derived aromatic hydrocarbon compounds modulate cancer progression. In this review, we discuss the findings of recent investigations addressing this issue.

Clinical Outcome in Relation to Smoking Status

An early study on patients with small cell lung cancer (SCLC) found that those who ceased smoking before chemotherapy or chemoradiotherapy had significantly better survival than those who did not.[7] A recent retrospective study also showed that persistent smoking during chemoradiotherapy adversely affected the treatment results against limited SCLC.[8] According to another study, 79 (42%) continuing smokers had significantly shorter survival (median 13.6 months) than 107 former smokers (18 months; $P = 0.0017$). Although the rates of toxicity-related treatment break and of non-cancer-related death were comparable, smokers with a treatment break exhibited the poorest survival (median 13.4 months). We speculated that cancer cells can be stimulated from tobacco-smoke-specific chemical compounds such as nicotine (to bombesine-like peptide receptor) and that smoking decreases cellular immunodefense during a therapeutic break.

On the other hand, it remains unclear whether a smoking status is a prognostic factor in patients with non-small-cell lung cancer (NSCLC). Tammemagi et

Reprint requests to: I. Yoshino
Received: October 5, 2006 / Accepted: January 21, 2007

al.[9] reported that a smoking history is an independent adverse factor for lung cancer survival on the basis of the findings of an analysis of 1155 patients (including 26% with SCLC and 24% with unknown histology) by adjusting several variables such as age, histology, and stage. They found that the hazard ratio of current smokers compared with never or former smokers was 1.37, and it was still 1.26 when 18 comorbidities were adjusted, which led them to conclude that the adverse effect of smoking is independent of smoking-related comorbidities. However, the design of their study is open to criticism in terms of the histological variety and definition of "current smoker" as smoking within 4 weeks. Toh et al.[10] reported that among Singaporean patients with advanced NSCLC, never smokers had a longer median survival than smokers (18.5 months; n = 117 vs. 13.6 months; n = 200), although the difference was not significant (P = 0.14) probably because of low statistical power. In their cases, the subpopulations were varied according to their smoking status: never smokers had a higher rate of adenocarcinoma (75%) and were mainly women (74%), both known to be subgroups with a good prognosis. A multivariate analysis could not show a significant impact of smoking status on survival. Similar results were also obtained from our retrospective analysis of 1123 Japanese patients with operable NSCLC.[11] In that series, female patients with adenocarcinoma, who were mainly non-smokers, showed a favorable prognosis than their counterparts. Recently, Tsao et al.[12] analyzed 1370 patients from the United States with stage III or IV NSCLC, and found that never smokers accounted for only 6% of the chemoradiation cohort, and 16% of the chemotherapy cohort. They reported an unfavorable outcome of smokers in the chemotherapy cohort but not in the chemoradiotherapy cohort. Moreover, responses to frontline chemotherapy among never, former, and current smokers differed significantly (19% vs. 8% vs. 12%, P = 0.004). A multivariate analysis revealed that stage and smoking status were the only factors predictive of response to chemotherapy, and the likelihood of response of former and current smokers was 0.38 and 0.57, when compared with never smokers. The 1-year survival rates of the never smokers, former smokers, and current smokers were 63%, 42%, and 43%, respectively (P < 0.0001). A multivariate analysis revealed that performance status, sex, and a prior smoking history were the only independent prognostic factors, and that the hazard ratio was 1.47 for former smokers and 1.55 for current smokers (P = 0.0004). In both cohorts, the current smokers had a comparable survival and therapeutic response than the former smokers. These investigators speculated that the reason for the effect of active smoking on therapeutic outcome in patients with advanced NSCLC may be fewer comorbidities and preserved lung function in never smokers, although no adjustment for such factors was considered in the study.

In all of these studies, there were more women and patients with adenocarcinoma in the never-smoker group; thus, the effects were analyzed by adjusting these factors. Nordquist et al.[13] examined the effect of smoking on lung adenocarcinoma and found that the smokers had a significantly unfavorable prognosis with only a 16% 5 year cancer-free survival rate versus a 23% 5 year cancer-free survival rate among never smokers.

In patients with earlier stage NSCLC, who may survive longer, persistent and active smoking seems to increase the chance of having second primary aerodigestive cancers during their clinical course.[14] The estimated rate of second primary cancers is 1%–4% per patient year, and the prevalence is high in patients with surgically cured, earlier stage NSCLC. Therefore, the therapeutic outcome may be more influenced by the smoking status in patients with operable NSCLC than in those with an advanced disease. Wu et al.[15] reported that never smokers had a significantly better cancer-specific survival rate 5 years after surgery (72%) than smokers (51%), although the cell-type or stage was not taken into account in that analysis. We also examined the impact of the smoking status on postoperative survival in 999 patients who underwent resection of NSCLC.[16] Among patients with adenocarcinoma, but not squamous cell carcinoma, the never smokers tended to have earlier stage disease and better survival than the heavy smokers. Furthermore, the impact of smoking status was recognized only in stage I adenocarcinoma. According to a multivariate analysis of survival, a smoking history (yes or no) and a pathologic stage (IA or IB) were the only independent factors, with respective hazard risks of 1.7 and 2.3. The rate of bronchioloalveolar carcinoma was not a factor.

In summary of these clinical analyses, smoking has an impact on the therapeutic outcome in both early and advanced NSCLC as well as SCLC, which is possibly attributable to the modulation of the biological behavior of lung cancer. However, the adverse effects of the accompanying comorbidities related to smoking habits, such as chronic obstructive pulmonary disease, interstitial pneumonia, and ischemic heart disease, might not have been excluded in these clinical studies. Therefore, we address the direct effects of tobacco-related chemicals on cancer cells, which support the hypothesis strongly.

Relationship Between Smoking History and Genetic or Biologic Markers

In this section, we discuss the genetic or biological modification that is the result of smoking. There are

more than 4000 chemical materials in tobacco smoke, and approximately 200 may be carcinogens, such as polycyclic aromatic hydrocarbons (PAH). During the development of lung cancer, the silencing of tumor-suppressor genes, such as loss of heterozygosity (LOH), methylation, and mutation, is the most important step. Moreover, the irreversible DNA adduct to benzo(a)pyrene diol epoxide (BPDE) and other PAH-diol epoxides, a metabolite of PAHs, is a prime factor of these DNA alterations. When the incidence of LOH was examined using five non-specific microsatellite markers for the NSCLC tissue, stage IA cases had a lower incidence of LOH, which was significantly associated with the smoking status.[17,18] In the bronchial biopsy specimens from volunteers, an examination by microsatellite markers for the regions of fragile histidine triad (FHIT), p53, and CDKN2, confirmed LOH in 76% of current smokers.[19] FHIT is a recessive oncogene, the expression of which is often altered in the structural and epigenetic arrangement during the early phase of lung carcinogenesis. In fact, 80% of current smokers with NSCLC have LOH at the locus of FHIT (Fig. 1), versus 22% of never smokers.[20] Methylation of the *FHIT* gene is also remarkably higher among chronic smokers than among never smokers at 45% versus 13%.[21] Maruyama et al.[22] recently reported that the methylation of FHIT was the only prognostic marker among eight genes tested for methylation status. The accumulation of such smoking-related genetic alterations would upgrade the malignant potential of lung cancer; however, the mechanism of LOH is not yet clear, although impairment of the process of DNA repair for PAH–DNA adduct formation may be involved.[23]

The mutation of oncogenes or tumor-suppressor genes is often recognized in patients with a smoking history. Recently, Tam et al.[24] reported that the KRAS mutation detected in 21 of 215 lung adenocarcinomas was significantly associated with smokers (11 of 58, $P = 0.003$), whereas mutation of the epidermal growth factor receptor (EGFR) detected in 115 of 215 adenocarcinomas was associated with non-smokers (83 of 111, $P < 0.001$). Interestingly, 75% of the mutations of KRAS were G:C to T:A transversion at codon 215 or 216, which is the best known mutation pattern in the gene related to smoke-induced oxidative stress.[25] Patients with the KRAS mutations had significantly worse survival after surgery than those without the KRAS mutations (32% vs. 70%, $P < 0.001$),[26] and gained no benefit from adjuvant chemotherapy.[27] With regard to the p53 mutations, exposure to smoke is also associated with G:C to T:A transversions in lung cancers,[28] and the incidence of p53 mutations was reported to be 47.5%, 55.6%, and 77.4% in never, former, and current smokers with lung cancers, respectively.[29] The impact of the incidence of the p53 mutations on the prognosis of patients remains controversial.[26,30]

It has been shown that patients who quit smoking substantially decrease their relative risk of contracting and dying of lung cancer within 5–15 years;[31,32] therefore, tobacco-related carcinogenesis may somehow be a reversible process. Tobacco-derived PAH can drive expression of various proteins via the aryl hydrocarbon receptor (AhR) or the nuclear factor (NF) κB, which promote genetic expressions of metabolism, inflammation, and proliferation. These molecular streams do not evoke a covalent reaction, so they are reversible to some extent. Zhang et al.[33] reported that the level of immunostaining of nuclear NFκB was associated with the expression status of cyclooxygenase-2 caspase-3 and p53, and that the expression level of nuclear NFκB was an independent prognostic factor.

We investigated the expression of several molecules related to the smoking status and prognosis in the NSCLC tissues. S-phase kinase-associated protein 2 (Skp2) is associated with degradation of p27, which is a cell-cycle regulator.[34] A high expression of Skp2 and a low expression of p27 are significantly related to a high grade of malignancy in various types of cancer, such as lymphoma, gastric cancer, and NSCLC.[35] In our recent study,[36] a logistic regression analysis for factors relating to the expression of Skp2 in NSCLC tissues revealed a strong positive relationship between the smoking history of patients and Skp2 expression in cancer tissues, with a hazard ratio of 12.9. Moreover, the level of Skp2 expression was an independent prognostic factor of pathologic stage in the Cox proportional hazard model analysis (Fig. 2). Type II hexokinase (HKII) and glycolytic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are glycolytic enzymes known to be upregulated in solid tumors.[37] When the RNA of the enzymes in NSCLC tissues was quantified, its expression levels were significantly correlated with postoperative prognosis and with smoking status,[38] whereas the expression of β-H^+-adenosine triphosphate (ATP)-synthase, which plays an important role in the Krebs cycle and generates ATP under aerobic conditions, was not. Macrophage migration inhibitory factor (MIF) is not only a classical chemotactic cytokine, but also a pituitary hormone, which acts to prolong inflammation as an antagonist to corticosteroids.[39] Recent attention has been paid to MIF as a key molecule linking inflammation to carcinogenesis.[40] We analyzed the expression of RNA for MIF in NSCLC tissues and found that it was well correlated with smoking status and prognosis in patients with squamous cell carcinoma.[41] The findings of these clinicopathologic studies suggest that the expression of the various molecules involved in the development of lung cancer can be upregulated by tobacco constituents, and may be reversible.

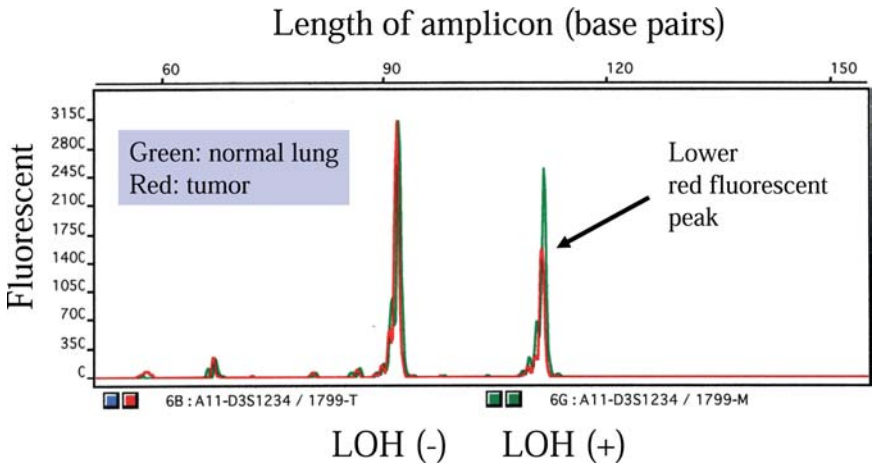

Fig. 1. Detection of loss of heterozygosity at the locus of fragile histidine triad gene using a marker of D3S1234 in lung adenocarcinoma in a smoker. There were two peaks among 92 base pairs and 105 base pairs: the tumor-derived peak (*red*) of the latter was lower than the normal lung-derived peak (*green*)

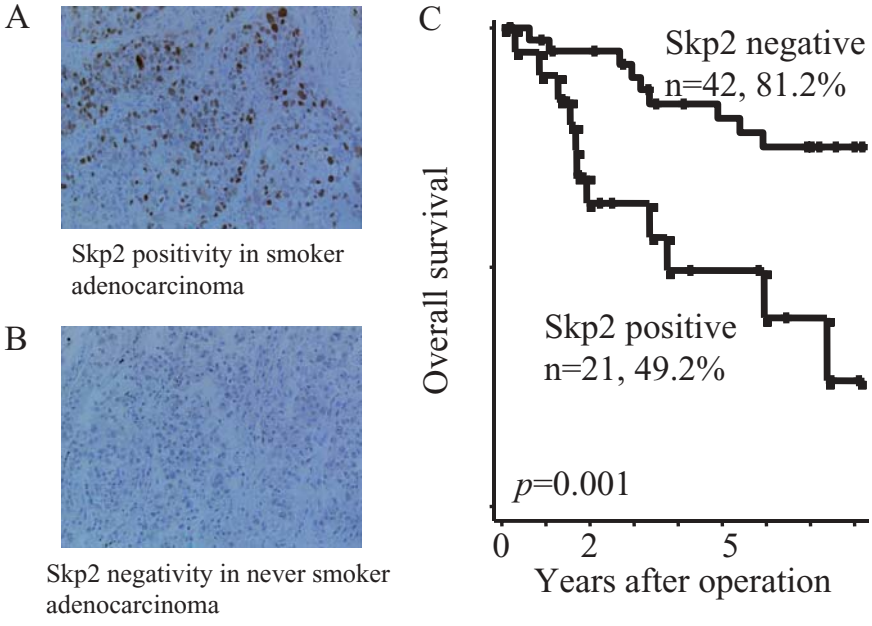

Fig. 2. Expression of S-phase kinase-associated protein 2 (Skp2) and postoperative prognosis in non-small-cell lung cancer (NSCLC). **A** and **B** Expression of Skp2 was preferentially detected in a smoker with lung adenocarcinoma. Representative cases are shown. **C** Survival curve for stage I NSCLC with high-or low-expression of Skp2

Analyses of the Effects of Tobacco Smoke on Cancer Cell Biology

Nakanishi et al.[42] reported that B(a)P treatment induced p53 and p21 expression in lung adenocarcinoma cells (A549), but they observed no apoptosis. This led them to speculate that simultaneous upregulation of the proteosomes and degradation of p21 abrogated the apoptotic signals. They also reported that PAH induce interleukin-8 through the activation of NFκB in A549.[43] Interleukin-8 is known to be an important angiogenic factor for NSCLC.[44] The mechanisms of the activation of NFκB are obscure, but it has been speculated that the complex of AhR and PAH interacts with the response element upstream *NFκB/Rel* gene, or that BPDE binds covalently to DNA, causing DNA damage, which in turn activates NFκB.[43]

Epidermal growth factor receptor is highly expressed and activated on the surface of various cancer cells. Recently, tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a known carcinogen, was found to be an agonist for β1- and β2-adrenergic receptors. It transactivates EGFR (dimerization, phosphorylation, and internalization) via the β-adrenergic system in pancreatic cells.[45] EGFR ligands, such as amphiregulin [46] and heparin-binding epidermal growth factor,[47] are shed from the cell membrane of the respiratory epithelium in response to tobacco-smoke. EGFR ligands also activate EGFR in an autocrine or paracrine manner. Transcription of cyclooxygenase-2 (COX-2), which is associated with the progression and poor prognosis of NSCLC through the synthesis of prostaglandin E2 (PGE2),[48] is also upregulated by tobacco-smoke induced EGFR activation in oral epithelial cells.[49]

Table 1. Highly upregulated gene expression in lung adenocarcinoma cells after B(a)P treatment

Genes	Function (possible role in tumor progression)	Fold expansion of gene expression[a]
Migration stimulating factor	Migration (invasion, metastasis)	38.9 (50.0)[b]
Plasminogen activator inhibitor type 1	Inhibition of fibrinolysis (invasion)	32.6 (25.6)
BCL2-related protein	Cell division (proliferation)	24.0 (14.1)
Fibronectin	Matrix, adhesion (invasion, metastasis)	21.3 (23.9)
Coagulation factor II receptor-like 1 precursor	Coagulation	16.1 (18.4)
Nectin-like protein	Adhesion to matrix (metastasis)	14.0 (11.0)
Immunoglobulin superfamily, member 4	Recognition	12.8 (9.0)
Amino acid transport protein	Synthesis	12.4 (15.0)
Proteinase-activated receptor-2	Proteolysis (invasion)	11.7 (10.7)
XAGE-1 protein	Development	10.3 (12.6)
GATA-binding protein	Nucleic acid metabolism	10.2 (7.7)

[a]The expression signal of B(a)P-treated A549 cells was divided by that of DMSO-treated A549 cells
[b]The expression signal of A549 cells cultured with B(a)P-free media following B(a)P exposure was divided by that of DMSO-treated A549 cells

Pulmonary fibroblasts were recently found to produce COX-2 and PGE2 in response to tobacco smoke.[50] In this way, tobacco-smoke components readily induce cell proliferation, inflammation, and cell migration.

We recently examined the effects of long-term (24 weeks) exposure to B(a)P in lung cancer cells (A549). A549 cultured with media containing 1 μM of B(a)P had higher proliferative activity than A549 cultured with media containing dimethyl sulfoxide (DMSO) (control). A microarray analysis of more than 20000 genes revealed irreversible changes of expression of 4470 and reversible changes of expression of 1021. The genes with greater than 10-fold upregulation in the B(a)P-treated A549 cells in comparison with the DMSO control are listed in Table 1. Of the 11 genes, 6 were related to cell migration, adhesion, or proliferation, and potentially, to the progression of cancer. Interestingly, these genes remained upregulated when A549 cells were cultured with B(a)P-free media for 8 weeks after exposure to B(a)P (Table 1). Migration stimulating factor, plasminogen activator inhibitor 1 (PAI-1), fibronectin, nectin-like receptor, and proteinase-activated protein have all been reported to play a role in the mobility of cells. One of these genes, the *PAI-1* gene, which was upregulated 32.6-fold (Table 1), was the most strongly implicated as it is a well known a key molecule of progression in various cancers, including lung cancer.[51] The plasminogen activation system is a widespread proteolytic mechanism, which participates in extracellular matrix degradation, thrombolysis, and cancer invasion.[52] Urokinase- and tissue-type plasminogen activators derived from various cancers play an important role in invasion, and a number of studies of clinical cancers have shown an association between high expression of plasminogen activator and a poor prognosis.[53] In a report by Pederses et al.[54] multivariate analysis showed that high expression of PAI-1 was significantly correlated with short survival but expression of the urokinase-type plasminogen activator gene was not. In our microarray analysis, the urokinase-type plasminogen activator was not upregulated (0.5-fold) by B(a)P treatment. In the other molecules listed in Table 1, matrix metalloproteinase (a well-known cancer invasion-related proteinase[55]), and NFκB (a nuclear factor known to be induced by B(a)P[56]), were upregulated 3.0-fold and 2.5-fold, respectively. EGFR and one of its ligands, epiregulin, were also upregulated 8.7-fold and 3.2-fold, respectively. This in vitro analysis may demonstrate the cellular phenomena behind the clinical situations.

Analyses of the Effects of Tobacco Smoke on Immunological Defense

It has long been accepted that tobacco smoke modulates the immune system.[57] Decreased humoral immunologic responses have been demonstrated clinically in smokers, by low serum levels of immunoglobulins other than IgE.[58] The cellular arm of adaptive immunity remains controversial in regard to whether smoking deteriorates T cell responses;[59] however, the molecular mechanisms of tobacco smoke-induced suppression of cellular immunity have recently been elucidated. Frazer-Abel et al.[60] reported that nicotine inhibits the proliferation of T lymphocytes through the activation of the NF of activated T cells c2 (NFATc2). The NFATc2 represses cyclin-dependent kinase 4 and increases, resulting in stabilized p27 and T lymphocyte G1-arrest. Tumor-derived COX-2/PGE2 is thought to be responsible for the altered immune network in various solid tumors.[61] Sharma et al.[62] reported that COX-2/PGE2 promotes expression of FoxP3 (a T regulatory cell-specific transcription factor), and activates T regulatory cells (a key population of T cells with immunosuppressor activity).

They also reported that the inhibition of COX-2 activity reversed the antitumor response in mice given PGE2. Previously, we observed substantial accumulation of CD25-positive CD4 T cells in clinical lung cancer tissues.[63] Moreover, the T cell phenotype has been elucidated to be regulatory T cells. Kalra et al.[64] reported that chronic exposure to nicotine impairs the phosphatidyl inositol 3-phosphate-sensitive calcium ion stores and suggested that antigen-mediating signaling is suppressed in smokers. Impaired presentation of tumor antigens is thought to be a major cause of abortion of the anti-tumor host defense. The number of dendritic cells, professional antigen-presenters, decreased significantly in the lung tissues of mice exposed to tobacco smoke.[65] Tumor-derived COX-2 is also involved in the suppression of dendritic cell activity in tumor tissues.[66] Anti-tumor T cell immunity in lung cancer is hardly recognized in vitro unless the T lymphocytes are stimulated with prepared autologous tumor cells.[63,67] The above mechanisms may underlie the cause of the anergic state of infiltrating lymphocytes in lung cancer tissues. The role of antitumor immunity in the treatment of lung cancer is still unclear; however, promising vaccine therapies are now under investigation.[68,69] A reverse treatment of tobacco-induced immunosuppression is probably needed for the success of immunotherapies against lung cancer.

Individual Differences in Susceptibility to Smoking Effects

The adverse effects of a smoking status on the clinical outcome in terms of reliability of the fact and its reasons remain very controversial, although tobacco-derived chemical compounds readily modulate cell function by altering genes and promoting various molecules. The most important considerations in the clinical study of the effects of smoking are the individual differences in susceptibility to these effects.

Recently, single nucleotide polymorphisms (SNPs) have been clarified for cytochrome 450P (CYP)1A1[70] which converts B(a)P to BPDE. The homozygosity of the CYP1A1*2 allele is associated with an increased risk of lung cancer in Asians, but this polymorphism is never, or rarely, recognized in Caucasians. The polymorphisms of DNA repair genes must also be considered. The polymorphism of glutathione S-transferase (GST) M1, which plays an important role in the detoxification of BPDE and other PAH-metabolites, strongly affects DNA damage and the risk of lung cancer developing in smokers.[71] The mean BPDE–DNA adduct level in persons with GSTM1*0 was 6.4 adducts, whereas it was 1.2 in those with GSTM1*1.[72] Alexandrov et al.[73] found that the combination of homozygosity of CYP1A1*2 and GSTM1*0 presents a high risk of PAH-metabolites in DNA adducts. Oxoguanine glycosylase 1 (OGG1) is glycosylase involved in the excision of 7, 8-dihydro-8-oxoguanine, a common oxidized guanine induced through oxidative stress by smoking.[74] Its gene also presents functional polymorphisms. The homozygous form of the ser326cys variant is found in about 10% of people and is responsible for decreased activity of OGG1.[75] The *XPD* gene (also called *ERCC2*) encodes a helicase that is part of the TFIH complex.[76] Several polymorphisms, including non-synonymous SNPs, at the 312 and 751 codons have been described and are associated with an impaired DNA repair capacity, thereby resulting in an increased risk of lung cancer.[77] These SNPs of the genes encoding the critical enzymes contribute to the susceptibility of lung cancer, and may be involved in the biological modulation of established lung cancer in smokers. Ultimately, the patients with lung cancer are more likely to have the genetic features in the mentioned enzymes than healthy persons.

Another problem when investigating the clinical effects of smoking is that there are no objective parameters or methods of brief evaluation of an individual's exposure to smoking, other than the pack-year index or the term abstinence from smoking. Various metabolites of tobacco smoke-derived chemicals, such as PAH, aromatic amines, and tobacco-specific nitrosamines, form the DNA adduct,[78] which is thought to be an essential early step in the development of cancers. A quantification of the DNA adduct in the respiratory cells may be the proper measure for the DNA damage caused by smoking. The ^{32}P-postlabeling assay is the classical method to measure DNA adducts of carcinogens.[79] Using this method, Boysen and Hecht[80] detected BPDE–DNA adducts in 45% of smokers, 33% of ex-smokers, 52% of non-smokers, 39% of occupationally exposed individuals, and 34% of environmentally exposed individuals. Recently, Arif et al.[81] attempted to measure PAH–DNA adducts by using a modified ^{32}P-postlabeling assay/thin layer chromatography, in which the adducts were eluted as diagonal radioactive zones from the purified lung DNA of patients with lung cancer, and chromatographed in urea-based solvent. However, although the method could quantify free radicals, aldehyde, and butadiene, it could not quantify polyaromatics. A novel alternative is required to achieve a precise quantification of the individual DNA adducts of smoking-related metabolites.

Treatment for Smoking-Enhanced Lung Cancer Behavior

If the mechanisms are shared among lung carcinogenesis and progression of established lung cancer, cessation

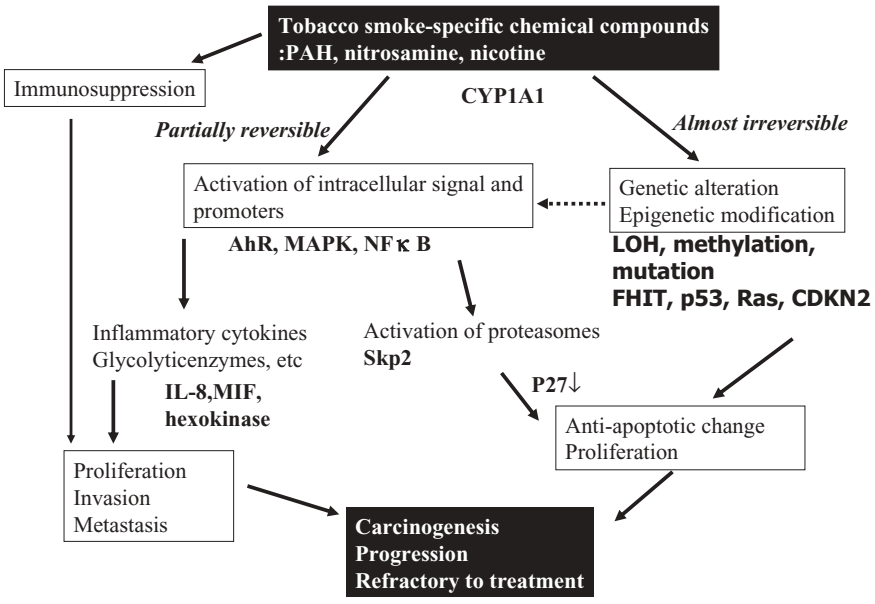

Fig. 3. Schema of the effect of smoking on the progression of lung cancer. Tobacco-related molecules such as benz(o)pyrene and nitrosourea are well-known carcinogens. These molecules have been shown to induce angiogenesis or inflammatory cytokines. Thus, we hypothesize that tobacco-related molecules may play an important role in the progression of established lung cancer as well as lung carcinogenesis

of smoking and chemoprevention may be effective for the treatment of smokers with lung cancer. Despite two decades of research into the chemoprevention of lung cancer, there have been no breakthroughs, and several studies even showed unexpected adverse results. Beta-carotene alone, with vitamin A or with vitamin E, was found to be associated with a significantly increased risk of lung cancer,[82,83] and 13-*cis*-retinoic acid had no benefit on the intervention, but it increased the risk of recurrence and mortality in smokers with stage I NSCLC.[84] On the other hand, in preclinical studies, the inhibition of 5-lipoxigenase[85] and of cyclooxygenase (COX)[86] decreased lung tumorigenesis by inhibiting the production of prostaglandins and leukotrienes, and COX-2 inhibitors suppressed the production of inflammatory cytokines and cell-cycle regulators by inhibiting the NFκB activation induced by tobacco smoke.[87] Inhibitors of these agents are currently the subjects of phase IIB studies to investigate whether bronchial dysplasia is reduced by the administration of these agents. Selenium, an anti-oxidative supplement, is also the subject of a phase III study, after being proved in decreasing the incidence of lung cancer by 44% in a secondary analysis of the Nutritional Prevention of Cancer, the primary endpoint of which was the effect of selenium on reducing non-melanoma skin cancers.[88] Recently, a bioflavonoid quercetin (3,3′,4′,5,7-pentahydroxyflavone), which is concentrated in various fruits and vegetables and exerts a cytostatic effect on tumors,[89] has been shown to reduce the level of PAH–DNA adducts and precancerous pathologic changes of the lung in mice.[90] Such agents may suppress the effects of smoking on lung cancer cells, and inhibit progression.

Conclusion

The smoking status is likely to affect the clinical outcome of both early and advanced stage lung cancers, and tobacco smoke-specific chemical compounds promote the progression of lung cancers via DNA alterations (which are possibly irreversible), and the modification of various protein expressions (some of which are possibly reversible; Fig. 3). This may be confirmed by clarification and evaluation of susceptibility to tobacco-derived carcinogens. All oncologists, especially thoracic oncologists, must consider the relationship between smoking and shorter survival, and advise smokers to stop smoking to achieve optimal treatment results. The mechanisms involved in the smoking-related progression of lung cancer must be investigated further to find therapeutic targets for lung cancer as tobacco smoking is the most "certain" predisposing cause of lung cancer death.

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