INVESTIGATION OF THE ASSOCIATIONS OF SMOKING-RELATED DNA DAMAGES WITH BIOMARKERS IN A HUMAN LUNG CANCER POPULATION

PhD theses

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Introduction

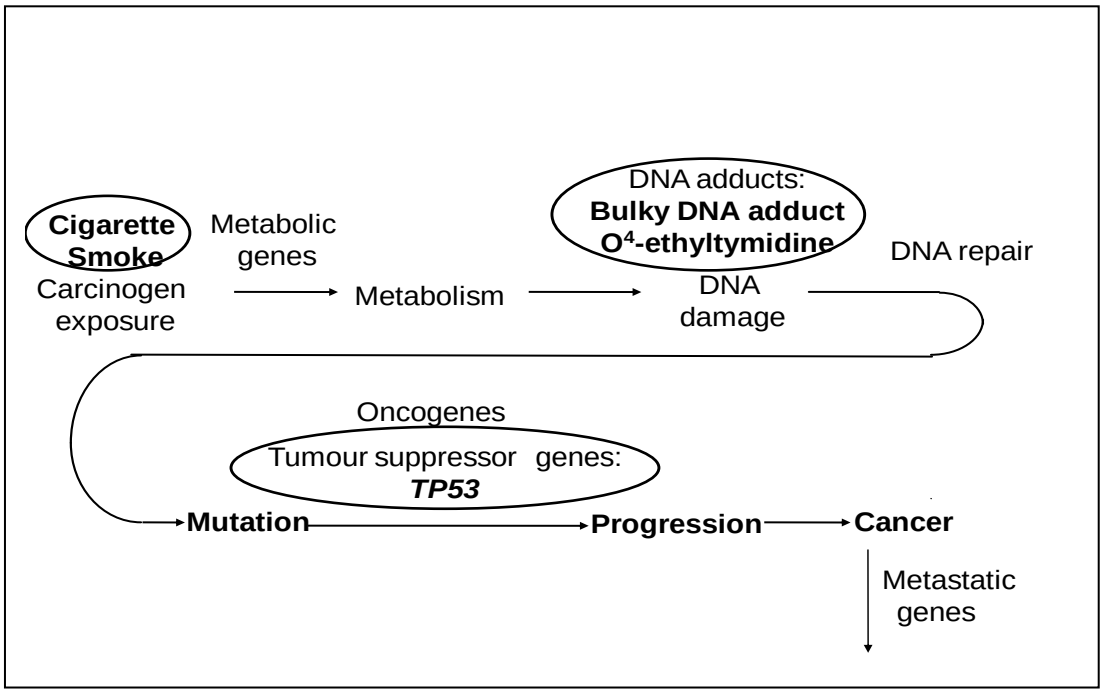
Lung cancer rate in Hungary is one of the highest in the world among men and also very high among women, for reasons not clearly understood yet. The primary risk factor for lung cancer is smoking. In Hungary, cigarette consumption is high, but not substantially different from the cigarette consumption of other much-smoking countries. The risks associated with other potential etiological factors, such as asbestos and radon exposure, are not known to be different in Hungary from those in many other European countries. Therefore, the complex molecular mechanisms of the disease should be further explored in order to reveal the reasons for the very high lung cancer rate in Hungary.

Tobacco smoke contains more than 4000 constituents including polycyclic aromatic hydrocarbons (PAHs), heterocyclic amines, N-nitrosamines and aldehydes, many of which are genotoxic. Some carcinogenic agents damage the DNA directly, but the majority of the carcinogenic agents undergo metabolic activation before they become biologically active. The biological markers, or briefly biomarkers, used in molecular epidemiology, are biological materials, components or processes that can be detected and measured in the human body; they reflect processes and changes in the organism, may influence the onset or outcome of a disease, or may indicate the risk of a disease. The new knowledge gained from biomarker research may facilitate the reduction of adverse health effects of human environmental exposure and the occurrence of certain diseases. The main groups of biomarkers that characterise the multi-step process of carcinogenesis are the exposure markers, such as DNA adducts, the effect markers, such as gene mutations, and susceptibility markers that may influence the whole process.

The complex molecular mechanisms of lung cancer can be investigated and characterised by the different types of biomarkers and by the associations among them. In my doctoral dissertation I have investigated the associations among bulky DNA adducts and O^4-ethyltymidine that are biomarkers of exposure, the *TP53* gene mutation spectra as a biomarker of effect, and smoking exposure as the main environmental risk factor of lung cancer.

Aims of the research

The aim of my multi-endpoint molecular epidemiological research was to further explore the molecular background of lung carcinogenesis in Hungarian lung cancer patients with the application of exposure and effect biomarkers. The research was based on the previous studies of our research team on smoking-related bulky DNA adduct formation. The scheme here below shows the main stages of the carcinogenic pathway initiated by cigarette smoke exposure, and the circles indicate the exposure and the biomarkers investigated in the project.



The scheme of the carcinogenic pathway initiated by cigarette smoke

I extended my investigations in the frame of international collaborative projects to a hardly-known type of DNA damage, O^4-ethylthymidine (O^4-etT) to explore whether it is suitable for use as biomarker of smoking exposure. Thus, a major aim of my research was to explore new exposure biomarkers in target tissue, here, specifically in human lung tissue. I analysed separately and in correlation the dose-dependency and the elimination of the two different types of DNA adducts in order to explore possible links between their formation and elimination pathways. For the first time, I investigated the *TP53* gene mutations in association with the smoking status and tumour histology in a Hungarian lung cancer population in order to compare the Hungarian characteristics with international statistics. The international novelty of my research is that this is the first human study in which the associations between

smoking-related bulky DNA adducts as primary DNA damage, and specific *TP53* tumour suppressor gene mutations as a possible consequence have been investigated for possible causal relationship that had been suggested by previous experimental model studies.

Materials and methods

Study population

Lung tissue samples derived from 104 primary lung cancer patients who underwent lung resection in the Department of Thoracic Surgery of the Korányi National Institute of Tuberculosis and Pulmonology (Budapest, Hungary). Macroscopically normal and tumour tissues samples were taken from the resected lobes, and DNA was isolated form the tissues. The study population comprised 37 squamous cell carcinoma and 67 adenocarcinoma cases. 60% of the cases were males (n=62) and 40% (n=42) females.

DNA isolation

DNA was isolated from the lung tissues samples by using the phenol – chloroform – iso-amyl alcohol extraction procedure.

Determination of bulky DNA adducts by ^{32}P-postlabelling

Bulky DNA adducts were determined in the genomial DNA from the non-tumorous (n=104) and tumour tissue samples (n=57) by using the ^{32}P-postlabelling method combined with nuclease P1 adduct enrichment. The radio-labelled DNA adducts were separated with multi-directional thin-layer chromatography. Detection of the adduct patterns and measurement of radioactivity were done by electronic autoradiography.

O^4-ethylthymidine determination with ^{32}P-HPLC method

O^4-etT levels were determined from the macroscopically normal lung tissue samples (n=64) with a modified immunoenriched ^{32}P-postlabelling method, followed by separation on reverse-phase high-performance liquid chromatography (HPLC) with gradient elution, and online detection of radioactivity.

TP53 mutation detection

TP53 mutations were analysed from the tumour tissues (n=104) in exons 5-9 and 11. The *TP53* gene sequences were amplified from the DNA samples by polymerase chain reaction (PCR). Denaturant gradient gel electrophoresis (DGGE) and automated capillary electrophoresis single strand conformation polymorphism (CE-SSCP) were applied to screen for *TP53* mutations. The mutations were determined by direct sequencing.

Statistical analyses

The statistical analyses were performed with GraphPad Prism 4.0 software, using Fisher's exact test and Mann-Whitney U-test, and Spearman correlation test. Two-sided P values are given, and a difference was considered statistically significant at $P \leq 0.05$.

Summary of the results

The aim of my PhD research was to further explore the associations among smoking status, two different DNA adduct types, the O^4-etT and bulky DNA adduct, the *TP53* tumour suppressor gene mutations and lung cancer in a molecular epidemiological study in a Hungarian lung cancer study population.

The levels of O^4-etT and bulky DNA adducts were significantly higher in the combined group of subjects who smoked until surgery or gave up smoking at most one year before surgery than in the combined group of those subjects who gave up smoking more than one year before the surgery or never smoked. O^4-etT appeared to be a highly persistent DNA damage. There was no statistically significant correlation between the individual levels of O^4-etT and of bulky DNA adducts.

The *TP53* mutation frequency and the variety of mutation types were higher in the present study population as compared to the IARC database. 45% of the samples carried *TP53* mutation. The mutation frequency was significantly higher in squamous cell carcinoma than in adenocarcinoma, and in the cases with more than 20 years of smoking history. The most common mutations were G→A (19%), G→T (19%) and G→C (16%) base changes. The mutation pattern was influenced by the smoking status. G→T transversion was detected

exclusively in smokers, and most carriers of the G→T transversions had also high level of bulky DNA adducts.

My results confirm that O^4-etT level is increased by smoking in the lung. O^4-etT is persistent in human lung, and the activation and elimination pathways of O^4-etT and bulky DNA adducts are not closely linked. I consider O^4-etT a suitable biomarker of smoking exposure for comparison of exposure groups in molecular epidemiological studies.

For the first time at international level, I demonstrated strong association between G→T mutation of *TP53* and high level of bulky DNA adducts in a human study, which is a significant scientific progress from the *in vitro* studies in the exploration of the causal relationship between a carcinogen-DNA adduct and a gene mutation.

Highlighted novel scientific findings

1. My results indicate that the main source of O^4-etT formation in human lung is smoking. O^4-etT is applicable in molecular epidemiological studies as a biomarker of smoking exposure.

2. My results demonstrate the long persistence of O^4-etT in human lung for several years after quitting smoking, contrary to bulky DNA adducts.

3. Although the major source of O^4-etT and bulky DNA adduct formation in lung is smoking, their metabolic pathways and DNA repair processes are probably not closely linked.

4. Higher *TP53* mutation frequency was found in the Hungarian lung cancer study population than in several Caucasian, South-American and Asian lung cancer populations, and its mutation frequency is among the highest ones published in the literature.

5. I found associations between *TP53* mutation frequency and gender, smoking dose, duration of smoking, and the histological type of tumour.

6. *TP53* mutation spectra were similar in never-smokers and in those former smokers who gave up smoking more than a year before surgery.

7. For the first time at international level, I demonstrated relationship between the high level of bulky DNA adducts and the smoking exposure-specific G→T transversion in human lung cancer.

Publications

Publications related to the PhD dissertation in scientific journals and book

Anna,L., Kovács,K., Győrffy,E., Schoket,B., Nair,J. (2011) Smoking-related O^4-ethylthymidine formation in human lung tissue and comparisons with bulky DNA adducts, Mutagenesis, 26, 523-527. **Impact factor: 3.98**

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30 International and national conference participations as first author with poster and/or oral presentations.
51 International and national conference participations as co-author with poster and/or oral presentations.

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