Traditional Approaches and New Perspectives in the Epidemiology and Prevention of Mutation-Related Diseases. The Frits Sobels Prize Lecture

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Introduction

The following article is a synthesis of the lecture given in the Island of Kos (Greece), on 3rd July 2005, as the recipient of the Frits Sobels Prize for 2005. This prize is awarded by the European Environmental Mutagen Society (EMMS) to senior scientists for major achievements in the field of environmental mutagenesis. It commemorates Professor Frits Sobels, founding President of EEMS and founding Editor of the journal Mutation Research.

Background

Kos is the island where Hippocrates, the Father of Medicine, was born and founded the well known Medical School. Fig. 1 shows the first sentence of the Hippocratic oath, a code of ethical principles
That is still administered in several countries, also including Italy, to graduates entering the medical practice. The sentence reports the names of Asclepius, the Greek God of Medicine, and of two of his daughters, Hygeia and Panacea. Panacea was the divinity who was worshipped by patients in order to recover from their diseases, while Hygeia was the divinity who was worshipped by healthy individuals in order to maintain their healthy status. Clearly, the word hygiene is etymologically derived from Hygeia, the goddess of health. The author is professor and chairman of Hygiene and Preventive Medicine at the School of Medicine of the University of Genoa. For 19 consecutive years, I served as the director first of the Institute of Hygiene and Preventive Medicine (1986 – 1998) and then of the Department of Health Sciences (1999 – 2005).

Within this discipline, I worked for 15 years in the area of epidemiology and prevention of infectious diseases, especially of viral diseases, such as paralytic polio, influenza, and viral hepatitis. In 1975, when I became professor, I took the difficult decision to change the focus of my research, and I started working in the areas of environmental mutagenesis, epidemiology and prevention of cancer and other chronic degenerative diseases. In 1978, almost simultaneously, I published a study in the Journal of Immunology dealing with hepatitis B virus and another study in Nature dealing with the metabolic deactivation of mutagens (2). The latter study anticipated my interests in the area of cancer chemoprevention. In fact, I demonstrated that several compounds whose mutagenicity tends to be detoxified by metabolic systems in vitro are not carcinogenic or are controversial carcinogens. This research line, supporting the existence of thresholds due to detoxification mechanisms, was expanded for some compounds. In the case of chromium(VI), we could estimate the amounts that are detoxified in different compartments of the human body (3). These patterns explain why chromium(VI) is carcinogenic to humans only in the respiratory tract, following inhalation of high doses overwhelming the body’s defense mechanisms.
The epidemiological revolution of the 20th century

The 20th century was characterized by a dramatic revolution of the epidemiological scenario. We were able to reconstruct, year by year from 1901 to 2000, the main causes of death in the Italian population, both in terms of raw mortality data (4) and of age-standardized data (5). In the meantime, life expectancy at birth almost doubled, from 42 years in 1901 to 80 years in 2000. Infectious and parasitic diseases were replaced by chronic degenerative diseases as prevailing causes of death in the population, with an impressive crossing of mortality curves by mid 20th century. A new turning point occurred during the last decades, with an evident decline of mortality for chronic degenerative diseases. For instance, by considering age-standardized data for both genders (5), in the year 2000 there was a decrease of mortality in Italy of 73% for cerebrovascular diseases, of 68% for cardiovascular diseases, and of 15% for tumours, compared to the maximum levels recorded during the 20th century. It is obvious that not only these diseases can be cured but they can also be prevented.

On the whole, the general mortality ratio in Italy fell from 30‰ in 1871 to 22‰ in 1901 and 9.7‰ in 2000. This means that, with the actual population size, 1,180,000 lives were saved every year as compared with 1871, and 1,276,000 lives were saved as compared with 1901 (6). These exciting data give an idea of the tremendous advancements due to the improvement of hygienic conditions and to the progress of medicine, both preventive and curative.
THE EPIDEMIOLOGICAL REVOLUTION OF THE 20th CENTURY

ITALY, RAW MORTALITY DATA

S. De Flora, A. Quaglia, C. Bennicelli & M. Vercelli, FASEB J. 19, 892–897, 2005

THE EPIDEMIOLOGICAL REVOLUTION OF THE 20th CENTURY

ITALY, AGE-STANDARDIZED MORTALITY DATA

S. De Flora, A. Quaglia, C. Bennicelli & M. Vercelli, FASEB J. 19, 892–897, 2005
**GENERAL MORTALITY IN ITALY**

<table>
<thead>
<tr>
<th>YEAR</th>
<th>POPULATION</th>
<th>MORTALITY</th>
<th>No. OF DEATHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1871</td>
<td>~26 millions</td>
<td>30ä</td>
<td>780,000</td>
</tr>
<tr>
<td>1901</td>
<td>~33 millions</td>
<td>22ä</td>
<td>726,000</td>
</tr>
<tr>
<td>2000</td>
<td>~58 millions</td>
<td>9.7ä</td>
<td>560,000</td>
</tr>
</tbody>
</table>

30ä 1,740,000 (Ğ1,180,000)

22ä 1,276,000 (Ğ716,000)

S. De Flora, A. Quaglia, C. Bennicelli & M. Vercelli, FASEB J. 19, 892–&897, 2005

**Prevention strategies**

The so-called black-box, represented in Figures 7 and 8 as a tunnel connecting multiple risk factors with multiple diseases, was extensively decoded in recent years. Intermediate biomarkers can be used in humans, experimental animals and wildlife organisms as indicators of the actual exposure load and of the potential risk to develop a disease. Their application can be useful for the purpose of both environmental hygiene and molecular epidemiology studies. In addition, they can be used to evaluate the efficacy of preventive interventions.

Fig. 9 explains the rationale of the three levels in cancer prevention. **Primary prevention** is addressed to apparently healthy individuals. Taking into account that the neoplastic mass and other lesions (e.g., atherosclerotic plaques) are of monoclonal origin, 30 cell doublings will be needed from a single cell to form a mass of 1 billion cells, approximately weighing 1 g. At this stage, sometimes it is possible to apply **secondary prevention**, which consists of early and possibly preclinical diagnosis, followed by timely intervention. Otherwise, in 10 divisions only, from the 30th to the 40th, the neoplastic mass will grow from 1 g to 1 kg. Diagnosis and suitable therapeutical treatments will be followed by rehabilitation and **tertiary prevention**. The latter consists of the prevention of local recurrences and of invasion and metastasis. The prevention of multiple primary cancers is another important target. As to primary prevention, the most obvious approach is avoidance of exposure to recognized risk factors. A complementary strategy is represented by chemoprevention, which involves the intake of protective factors and modulation of the host defence mechanisms by pharmacological or dietary agents, with the goal either of inhibiting carcinogenesis, which falls within primary prevention, or of reversing it at a premalignant stage, which falls in the boundary of secondary prevention.

MUTATION RESEARCH SPECIAL ISSUES ON PREVENTION OF MUTATION AND CANCER

S. De Flora (Ed.) “Role and mechanisms of inhibitors in prevention of mutation and cancer”

S. De Flora, G. Bronzetti and F.H. Sobels (Eds.)
“Assessment of antimutagenicity and anticarcinogenicity: end-points and systems”

L.R. Ferguson, G. Bronzetti and S. De Flora (Eds.)
“Mechanistic approaches to chemoprevention of mutation and cancer”
Prevention of Lung Tumors in Mice

Number of Tumors/Mouse

- Controls (Standard Diet): 0.35
- Urethane (Standard Diet): 11.06
- Urethane (Diet with NAC 0.2%): 1.95

S. De Flora et al., Cancer Lett. 32, 235-241, 1986
It is important not only to assess safety and efficacy of putative chemopreventive agents but also to understand the mechanisms involved. For more details on this subject I refer to three Mutation Research special issues (10). Fig. 11 reports a detailed classification of mechanisms, from inhibition of mutation and cancer initiation, either in the extracellular environment or in nontarget cells or in target cells, to inhibition of tumour promotion, progression, invasion and metastasis.

Fig. 12 shows an example of how it is possible to inhibit the formation of chemically induced tumours in mice by supplementing the diet with a chemopreventive agent. NAC (N-acetylcysteine) is a precursor of L-cysteine and GSH (reduced glutathione).

**Molecular alterations in chronic degenerative diseases and critical periods of life**

Although no generalization can be made, due to the huge number of clinical forms, it is noteworthy that different chronic degenerative diseases may share common risk factors, common protective factors, and common pathogenetic mechanisms, such as DNA damage, oxidative stress, and chronic inflammation (13). At equivalent exposure dose, the molecular dose, determined by the presence of DNA adducts (14), is affected by a considerable interindividual variability. Unless removed, DNA adducts can trigger initiation of cancer. For instance, as shown in Fig. 15, the levels of $^{32}$P postlabeled DNA adducts are remarkably enhanced in the liver of hepatitis virus (WHV)-infected woodchucks that are prone to develop primary hepatocellular carcinoma. WHV is a model for the human HBV. A series of studies performed in our laboratory provided evidence that the metabolic activation of hepatocarcinogens is enhanced in hepatocytes infected with either WHV or HBV.
DOSIMETRY OF XENOBIOTICS

EXPOSURE DOSE

PHARMACOKINETIC DOSE

CELLULAR DOSE

TARGET DOSE

MOLECULAR DOSE

TARGET CELL

INITIATED CELL

NEOPLASTIC CELL

DNA ADDUCT

ADDUCTS TO THE LIVER DNA OF WOODCHUCKS, AS RELATED TO INFECTION WITH HEPATITIS VIRUS (WHV)

WHV –

WHV +

A. Izzotti et al., Chem.-Biol. Inter. 97, 273-285, 1995
Similar molecular alterations can systematically be detected in smooth muscle cells composing the medium layer of abdominal aorta from atherosclerotic patients, where DNA adduct levels are correlated with occurrence of atherogenic risk factors known from traditional epidemiology (16). In addition, bulky DNA adducts accumulate with age in heart and, both in humans and in rodents, are increased following exposure to DNA-binding agents, such as those present in cigarette smoke (17). They are further increased by the combined exposure of rats to alcohol and smoke (18).

Oxidative DNA damage (8-OH-dG) is enhanced in the trabecular meshwork from glaucoma patients and, in both groups of controls and glaucoma patients, it is higher in subjects with a GSTM1-null genotype (19).

Compared to foetuses, in the lung of newborn mice there is a 5-fold increase of bulky DNA adducts and a 2-fold increase of 8-OH-dG, which is compensated by overexpression of many genes involved in stress response, antioxidant activity, and other protective mechanisms. In any case, administration of NAC to pregnant dams prevents these molecular alterations (20).

Mitochondrial DNA (mtDNA) is our biological clock that plays a crucial role in ageing and age-related pathological conditions, especially in postmitotic tissues having a high energy requirement. In the lung of smoke-exposed rats, the levels of adducts to mtDNA were even higher than those to nuclear DNA. These molecular alterations were considerably attenuated by the oral administration of NAC (21).

Immortalized human mammary epithelial stem cells were more susceptible than neoplastically transformed cells to the formation of bulky DNA adducts and 8-OH-dG following in vitro treatment with 7,12-dimethylbenz(a)anthracene (DMBA). Co-treatment of cells with NAC inhibited the induction of DNA changes (22).

Molecular Epidemiology of Atherosclerosis

The levels of \(^{32}\text{P}\) postlabelled DNA adducts in the aorta from 85 atherosclerotic patients were significantly correlated with:
- Age of patients
- Number of cigarettes smoked currently
- High blood pressure
- Blood triglycerides
- Blood cholesterol (total/HDL)
- SFS-positive DNA adducts
- Oxidative DNA damage (8-OH-dG)

S. De Flora et al., FASEB J. 11: 1021-1031, 1997
DNA ADDUCTS IN RAT ORGANS

Oesophagus
Liver
Lung
Heart
Controls Ethanol Smoke Smoke + Ethanol

A. Izzotti et al., FASEB J. 12, 753-758, 1998
8-OH-dG LEVELS IN THE EYE TRABECULAR MESHWORK

\[
\begin{array}{ccc}
\text{GSTM1} - \text{null} & \text{GSTM1} + \\
\text{Controls (n = 47)} & \\
\text{GSTM1} - \text{null} & \text{GSTM1} + \\
\text{Glucoma patients (n = 39)} & \\
\end{array}
\]

BIRTH–RELATED GENOMIC AND TRANSCRIPTIONAL CHANGES IN MOUSE LUNG

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DNA adducts ( / 10^6 ) nucleotides</th>
<th>8-OH-dG ( / 10^6 ) nucleotides</th>
<th>Expression of 746 genes (Newborn mice / fetuses)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UNTREATED DAMS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetuses</td>
<td>0.5 ± 0.03</td>
<td>1.9 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>Newborn Mice</td>
<td>2.5 ± 0.13 ( (P&lt;0.001) )</td>
<td>3.6 ± 0.33 ( (P&lt;0.05) )</td>
<td></td>
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<tr>
<td><strong>NAC–TREATED DAMS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetuses</td>
<td>0.4 ± 0.12</td>
<td>1.8 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>Newborn Mice</td>
<td>0.8 ± 0.09</td>
<td>1.9 ± 0.23</td>
<td></td>
</tr>
</tbody>
</table>
ADDUCTS TO LUNG DNA IN SMOKE–EXPOSED RATS

R. Balansky et al., Cancer Res. 56, 1642-1647, 1996

STEM CELLS
SUSCEPTIBILITY TO GENOTOXIC AGENTS
AND CHEMOPREVENTION

S. De Flora, S. Scarfi, A. Izzotti, F. D’Agostini, C.–C. Chang, M. Bagnasco,
A. De Flora and J. E. Trosko, 2005, submitted for publication
Transplacental effects of smoking

Active smokers are exposed to undiluted mainstream smoke, while involuntary smokers are exposed to environmental cigarette smoke (ECS), resulting from the mixture of sidestream smoke and the portion of mainstream smoke exhaled by active smokers (23). A further possibility of passive smoking is represented by the transplacental exposure during pregnancy (24). As shown in Fig. 25, the oral administration of NAC during pregnancy resulted in overexpression of only 3 of the 746 genes tested in foetus liver and, namely, two GSH S-transferase (GST) isoenzymes and an antitrypsin precursor playing a protective role in emphysema. Exposure of dams to ECS caused the upregulation of 117 genes, which were reduced to 42 when the ECS-exposed dams were treated with NAC. Thus, NAC met the criteria postulated for an optimal chemopreventive agent, which should not excessively alter the baseline expression of genes, as a molecular indicator of safety, but at the same time it should be able to inhibit carcinogen-related alterations, as an indicator of efficacy.

The ECS-upregulated genes included, among others, genes downregulating the cell cycle and genes stimulating apoptosis, which may explain hypoplasia of organs and growth retardation in the foetus. The observed overexpression of genes involved in leukocyte proliferation is consistent with a possible increase of leukemias and lymphomas in children from mothers smoking during pregnancy (26).

Systemic damage produced by cigarette smoke and light in hairless mice

Exposure of SKH-1 hairless mice to ECS, in a smoking machine, caused molecular, biochemical and cytogenetical alterations not only in skin but also in the respiratory tract, bone marrow, and peripheral blood (27). Exposure to the light emitted by UV-C-covered halogen bulbs exerted a variety of alterations in the skin, consistently with the potent carcinogenicity of these lamps (28). In addition, the light alone increased the levels of bulky DNA adducts in both lung and bone marrow and produced cytogenetical alterations in bone marrow and peripheral blood (29). Thus, UV-A- and UV-B-containing light, simulating solar irradiation, can produce a systemic genotoxic damage, which is presumably due to formation in the skin of sufficiently long-lived DNA-damaging derivatives, such as reactive aldehydes. In addition, exposure of mice to light potentiated the adverse effects observed in the respiratory tract of ECS-exposed mice (30). Administration of the nonsteroidal antiinflammatory drug sulindac to mice exposed to both light and ECS exerted a variety of protective effects (31).

All these data were further supported by the results of multigene expression analysis by cDNA arrays (32). In fact, the light alone upregulated 2 genes (GST and catalase) in mouse lung and increased the number of ECS-upregulated genes. Sulindac attenuated gene overexpression.
ACTIVE SMOKING AND PASSIVE (INVOLUNTARY) SMOKING

MAINSTREAM SMOKE

SIDESTREAM SMOKE

ENVIRONMENTAL CIGARETTE SMOKE (ECS)

PREGNANT MOTHERS: PLEASE DON'T SMOKE!

If you are pregnant or planning a family, here are three good reasons to quit smoking now:

1. Smoking retards the growth of your baby in your womb.
2. Smoking increases the incidence of infant mortality.
3. Your family needs a healthy mother.

Please don't smoke for your baby's sake. And yours.

AMERICAN CANCER SOCIETY
EXPRESSION OF 746 GENES IN MOUSE FETUS LIVER
(A. Izzotti et al., FASEB J. 17, 126-129, 2003)

TREATMENT OF DAMS DURING PREGNANCY

EXPRESSION OF 746 GENES IN MOUSE FOETUS LIVER
(DISREGULATION = 117 genes, 15.7 %)

A. Izzotti et al., FASEB J. 17, 126–129, 2005
SIGNIFICANT EFFECTS OF SMOKE IN HAIRLESS MICE

R. Balansky et al., Carcinogenesis 24, 1525–1532, 2003

Skin
• Bulky DNA adducts
• Oxidative DNA damage (8–OH–dG)
• Apoptosis (borderline)
• Increase of malondialdehyde

Respiratory tract
• Micronucleated and polynucleated pulmonary alveolar macrophages
• Proliferation (PCNA) and apoptosis in bronchial epithelium
• Bulky DNA adducts in lung
• Oxidative DNA damage (8–OH–dG) in lung
• Increase of malondialdehyde

Bone marrow
• Micronucleated polychromatric erythrocytes (PCE)
• Toxicity (decrease of PCE/NCE ratio)

Peripheral blood
• Time–related increase of micronucleated normochromatric erythrocytes (NCE)

SIGNIFICANT EFFECTS OF LIGHT IN HAIRLESS MICE

R. Balansky et al., Carcinogenesis 24, 1525–1532, 2003

Skin
• Thymine dimers
• Oxidative DNA damage (8–OH–dG)
• p53 oncoprotein
• Proliferation (PCNA)
• Apoptosis
• Increase of malondialdehyde

S. De Flora, F. D’Agostini, Nature 356, 569, 1992; Cancer Res. 54, 5081–5, 1994
SIGNIFICANT EFFECTS OF LIGHT IN HAIRLESS MICE
R. Balansky et al., Carcinogenesis 24, 1525–1532, 2003

Skin
- Thymine dimers
- Oxidative DNA damage (8-OH-dG)
- p53 oncoprotein
- Proliferation (PCNA)
- Apoptosis
- Increase of malondialdehyde

Respiratory tract
- Bulky DNA adducts in lung

Bone marrow
- Bulky DNA adducts
- Micronucleated polychromatic erythrocytes (PCE)

Peripheral blood
- Time–related increase of micronucleated normochromatic erythrocytes (NCE)

SIGNIFICANT EFFECTS OF LIGHT IN SMOKE–EXPOSED HAIRLESS MICE
R. Balansky et al., Carcinogenesis 24, 1525–1532, 2003

Respiratory tract
- Increase of malondialdehyde
- Bulky DNA adducts in lung
- Micronucleated pulmonary alveolar macrophages
SIGNIFICANT PROTECTIVE EFFECTS OF SULINDAC (NSAID) IN HAIRLESS MICE EXPOSED TO BOTH SMOKE AND LIGHT

R. Balansky et al., Carcinogenesis 24, 1525–1532, 2003

Skin
- Bulky DNA adducts
- Proliferation (PCNA)
- Malondialdehyde

Respiratory tract
- Micronucleated and polynucleated pulmonary alveolar macrophages
- Proliferation (PCNA) in bronchial epithelium (borderline)
- Bulky DNA adducts in lung
- Oxidative DNA damage (8-OH-dG) in lung
- Malondialdehyde

Bone marrow
- Toxicity (PCE/NCE ratio)

EXPRESSION OF GENES IN MOUSE LUNG (SKH-1)

Sham | Light | Smoke | Light + Smoke | Light + Smoke + Sulindac

| 2 (0.3%) | 24 (3.2%) | 29 (3.9%) | 11 (1.5%) |

Overexpressed genes out of 746 genes, tested by Atlas™ Mouse Stress (see Figure) and Expression cDNA arrays

A. Izzotti et al., FASEB J. 18, 1559–1561, 2004
Molecular alterations produced by environmental cigarette smoke in rats and their chemoprevention

As shown in Fig. 33, the whole-body exposure of Sprague-Dawley rats to ECS resulted in a variety of alterations, including formation of bulky DNA adducts in bronchoalveolar lavage (BAL) cells, tracheal epithelium, lung, heart, and thoracic aorta; oxidative DNA damage in lung; haemoglobin adducts of 4-aminobiphenyl and benzo(a)pyrene diol epoxide; micronucleated (MN) and polynucleated (PN) pulmonary alveolar macrophages (PAM); MN polychromatophilic erythrocytes (PCE) in bone marrow; proliferation and apoptosis in PAM. The following chemopreventive agents were administered, either with the diet or in the drinking water, to ECS-exposed rats: oltipraz (OPZ), 1,2-dithiole-3-thione (DTT), N-acetyl-L-cysteine (NAC), phenethyl isothiocyanate (PEITC), 5,6-benzoflavone (BF), and a combination of NAC with OPZ. With the exception of OPZ and, in part, of DTT, which even increased the frequency of MN PAM, all other chemopreventive agents significantly attenuated most ECS-induced alterations. PEITC stimulated apoptosis in PAM, while NAC inhibited apoptosis due to the upstream block of stimuli triggering this process (33).

In a further study, Sprague-Dawley rats, either untreated (SHAM) (34) or exposed to ECS (35), were treated with NAC, OPZ, BF, PEITC, and indole-3-carbinol (I3C), and combinations of OPZ with NAC and of PEITC with I3C. All treatments inhibited the formation of ECS-induced bulky DNA adducts in lung by at least 50%. The expression of 4,858 genes was analyzed in both liver and lung of rats. The hierarchical cluster generated in the lung of SHAM-exposed rats (34) showed that NAC is the closest treatment to SHAM, while the combination PEITC + I3C is the most disruptive treatment compared with the baseline gene expression. On the other hand, the same treatment was the farthest one from expression profiles recorded in ECS-exposed rats (35). Thus, the situation is complicate because it appears that the agents that most effectively change gene expression profiles in ECS-exposed rats do not necessarily revert them to the baseline situation.
4,858 GENES IN THE LUNG OF SHAM-EXPOSED RATS


4,858 GENES IN THE LUNG EFFICACY

Role of oncosuppressor genes in smoke-related carcinogenesis, and their modulation

Use of mouse strains deficient in oncosuppressor genes may be useful to clarify their mechanisms and to assess the efficacy of chemopreventive agents. Studies performed in mice having an A/J background and carrying a dominant \( P53 \) mutation in germ cells showed that (a) \( P53 \) plays a physiological protective role and (b) \( P53 \) mutant mice have an increased susceptibility to molecular, cytogenetic, and tumorigenic effects of cigarette smoke (36).

The \( Fhit \) gene is thus far the only example of a gene at a constitutive fragile region (3p14.2) and shows many hallmarks of a tumour suppressor gene. As shown in Fig. 37, only a small proportion of rat bronchial epithelial cells and kidney tubular epithelial cells do not have Fhit protein detectable by immunohistochemistry. Exposure of rats to ECS resulted, after only 28 days and in apparently healthy tissues, in a significant loss of Fhit in the bronchial epithelium. Of the tested chemopreventive agents (the same listed in Figures 34 and 35), only NAC was successful to slightly but significantly inhibit the ECS-related loss of Fhit (37).

Prevention of cancer progression

It is possible not only to prevent cancer initiation and promotion but also to inhibit advanced stages of the carcinogenesis process, such as progression, invasion and metastasis (see Figures 9 and 11). Inhibition of tumour angiogenesis provides an important strategy for cancer prevention and therapy (38), because the supply of oxygen and nutrients to the neoplastic mass becomes autonomous from surrounding tissues and because malignant cells can invade blood and lymphatic vessels.

In collaboration with Dr. A. Albini and colleagues from IST (Genoa), we demonstrated that NAC is able to inhibit VEGF and angiogenesis. For instance, the oral administration of NAC sharply prevented the vascularization of matrigel sponges, containing angiogenic factors, implanted subcutaneously in mice (39). In addition, oral NAC inhibited the growth of Kaposi’s sarcoma in nude mice and extended their lifespan, and in some cases determined a total regression of the tumour (40).

Chemoprevention trials in humans. Pharmacogenomic approaches

We participated to phase II chemoprevention trials in Italy, The Netherlands, and the People’s Republic of China. Fig. 41 shows that, in Dutch smokers, administration of NAC for 6 months significantly decreased the levels of both bulky DNA adducts and 8-OH-dG in BAL cells and the frequency of micronuclei (MN) in mouth floor cells.

There is an evident gap between the encouraging results obtained with many potential cancer chemopreventive agents in preclinical models and the negative or even disappointing results generated in phase I clinical trials. I suspect that there may be a large interindividual variability not only in susceptibility to mutagens and carcinogens but also in response to chemopreventive agents. As an example, Fig. 42 shows that the response to NAC, in terms of MN frequency (see Fig. 41), was amplified in smokers who were slow \( NAT2 \) acetylators or had a \( GSTM1 \) null genotype. This is a very preliminary result, and observations of this type need to be confirmed in further studies.
MOLECULAR ALTERATIONS AND LUNG TUMORS IN P53 MUTANT MICE, EITHER UNTREATED OR EXPOSED TO CIGARETTE SMOKE

(S. De Flora et al., Cancer Res. 63, 793-800, 2003; *A. Izzotti et al., Cancer Res. 64, 8566–72, 2004)

PHYSIOLOGICAL PROTECTIVE ROLE OF P53 IN A/J MICE

- Impairment of body weight gain (males only)
- Early changes of multigene expression in lung*
- Early stimulus of bronchial cell proliferation
- Age–related formation of bulky DNA adducts in both heart and lung
- Age–related increase of micronuclei in red blood cells (males only)

INCREASED SUSCEPTIBILITY OF P53 MUTANT MICE TO SMOKE

- Early upregulation of the expression of p53, k–ras and several other genes in lung*
- Increased formation of bulky DNA adducts in both heart and lung
- Increased frequency of micronuclei in pulmonary alveolar macrophages
- Increased frequency of micronuclei in red blood cells
- Increased incidence and multiplicity of lung tumors

LOSS OF FHIT EXPRESSION IN SPRAGUE-DAWLEY RATS EXPOSED TO CIGARETTE SMOKE FOR 28 DAYS

1.4 ± 0.74% 4.9 ± 1.96% 3.1 ± 1.7%
P < 0.001 compared to SHAM P < 0.05 compared to ECS

“Angioprevention” in multistage tumorigenesis

F. Tosetti et al., FASEB J. 16, 2-14, 2002

VASCULARIZATION OF MATRIGEL SPONGES, 72 HOURS AFTER SUBCUTANEOUS IMPLANTATION IN MICE

T. Cai et al., Lab. Invest. 79, 1151-1159, 1999
Conclusions

The herein reported results suggest that different chronic degenerative diseases may share similar pathogenic mechanisms, which is the premise to similar preventive approaches. It is meaningful that the dietary measures recommended for the prevention of cardiovascular diseases are essentially the same that are exploitable for cancer prevention. Chemoprevention is a well established strategy not only for the prevention of infectious diseases, for instance by using antiviral, antibacterial or antiprotozoan agents, but also for the prevention of certain chronic degenerative diseases. Thus, in order to prevent cardiovascular diseases, it is widely accepted to try to lower the arterial pressure or blood cholesterol or homocysteine first by means of dietary measures and then by using a pharmacological approach. A variety of dietary and pharmacological agents are potentially available for cancer chemoprevention. However, while it is easy to measure arterial pressure or blood constituents, it is necessary to find suitable molecular indicators that may be able to predict the efficacy of specific interventions for cancer prevention.

Acknowledgements

I thank all the collaborators in my laboratory (43) and a number of colleagues, from many countries (44), who have contributed to our studies.

Most of the cited studies were supported by the US NIH (National Cancer Institute) or by the Associazione Italiana per la Ricerca sul Cancro (AIRC).
### PHASE II CHEMOPREVENTION TRIAL WITH NAC IN DUTCH SMOKERS

<table>
<thead>
<tr>
<th>End-point</th>
<th>NAC Group (Mean ± SE)</th>
<th>Placebo Group (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₀</td>
<td>T₆</td>
</tr>
<tr>
<td>Cotinine in plasma [µM/L]</td>
<td>445 ± 57</td>
<td>467 ± 56</td>
</tr>
<tr>
<td>Cotinine in BAL fluid [µM/L]</td>
<td>1788 ± 315</td>
<td>3678 ± 1793</td>
</tr>
<tr>
<td>Urine genotoxins [x 1000 revertants]</td>
<td>154 ± 36</td>
<td>133 ± 18</td>
</tr>
<tr>
<td>PAH-DNA adducts in mouth floor cells</td>
<td>0.04 ± 0.01</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>PAH-DNA adducts in buccal mucosa cells</td>
<td>0.06 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>DNA adducts in blood lymphocytes</td>
<td>1.5 ± 0.1</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>DNA adducts in BAL cells</td>
<td>6.0 ± 0.9</td>
<td>4.3 ± 0.8</td>
</tr>
<tr>
<td>8-OH-dG in BAL cells</td>
<td>4.9 ± 0.7</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Micronuclei in mouth floor cells [%]</td>
<td>1.3 ± 0.3</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>Micronuclei in soft palate cells [%]</td>
<td>1.3 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>

Statistically significant as compared to T₀.

F.J. van Schooten et al., Cancer Epidemiol. Biomarkers Prev. 11, 167-175, 2002

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### PHARMACOCOGENOMICS / NUTRIGENOMICS OF CHEMOPREVENTIVE AGENTS

![Graph showing MN (‰) before and after NAC for different genotypes](image)

- **before NAC**
- **6 months after NAC**

- **NAT2**
  - Fast
  - Slow

- **GSTM1**
  - +
  - Null

- **All subjects**

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THE 100 SCIENTIFIC COLLABORATIONS