The relationship between genetic and environmental factors

La relazione tra fattori genetici e ambientali

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Riassunto: Il presente lavoro passa in rassegna i risultati conseguiti dalla ricerca epidemiologica in ambito biomolecolare relativamente all’associazione tra esposizioni ambientali e malattia. In particolare si discutono sostanze cancerogene e danni all’integrità strutturale del DNA. Questi consistono in un legame tra cancerogeno o un prodotto dell’interazione tra cancerogeno a processi cellulari e DNA che prendono il nome di addotti al DNA. Vengono poi presentati alcuni esempi relativi al potenziale predittivo degli addotti al DNA per lo sviluppo di tumore del polmone a seguito di esposizioni ambientali e le problematiche relative allo studio della interazione con la dieta e la suscettibilità genetica.

Keywords: Molecular Epidemiology, DNA Adducts, Cancer, Diet

1. Introduction

A number of diseases, including cancer, diabetes, cardiovascular and inflammatory diseases, are long term and undermine health, cause suffering and, in some case, shorten life expectancy. Most of them results from gene-environment interaction, but, while the "gene" is inborn, the "environment" is largely determined by lifestyle choices. Diet and nutrition are important components of lifestyle choices, and their role in the maintenance of good health and as determinants of certain types of human diseases has been established (WHO (2002)).

Now, at the beginning of the 21st century, the weight of evidence supports the notion that exposure to most environmental carcinogens results in damage to the structural integrity of DNA, which occurs primarily as covalent carcinogen binding and is referred to as DNA damage production (Pfeifer et al. (2000)). But, there is also growing evidence that endogenously generated compounds play an important role in the carcinogenic process, especially through the formation of endogenous DNA adducts (Bartsch et al. (1999), Munnia et al. (2004)). Indeed, a major development of the recent research has been the discovery that a significant amount of DNA lesions is arising from natural sources and

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that the endogenous production of DNA damage is involved in the aetiology of a number of cancers. The last years have known the development of several techniques focused on the measurement of DNA damage. Previously, for DNA damage analysis was necessary the administration of labelled chemicals and the analysis of DNA adducts in subjects was extremely rare. Over the last two decades, the development of analytical techniques has made it possible to assess DNA adduct production. The most used laboratory techniques are immunoassays and immunohistochemistry using DNA adduct specific antisera, $^{32}$P-postlabelling, fluorescence and phosphorescence spectroscopy, electrochemical detection and mass spectrometry (MS). The sensitivities of each assays is different and depend on the amount of DNA necessary that can be analyzed, but some techniques, such as the $^{32}$P-postlabelling assay, may detect up to one modified DNA adduct per $10^{10}$ normal nucleotides

2. Environmental exposures

A major achievement of molecular epidemiology has been its application to occupational epidemiology studies to analyze the effects of environmental exposures to carcinogens and to identify health hazards, especially by measuring the levels of DNA adducts, considered to be a reliable marker of the "biologically effective dose", in targets and surrogate targets of cancer disease (F.P (2000); Peluso et al. (2001); Perera et al. (2002)). Most of molecular epidemiology studies showed that the levels of DNA adducts tended to be higher among individuals heavily exposed to air pollutants (Peluso et al. (2001)). A meta-analysis of 13 DNA adducts studies, by the $^{32}$P-DNA postlabelling technique, on occupational cohorts exposed to air pollutants, showed that the association between DNA adduct levels and air pollutants was significant both in heavily exposed industrial workers and in less exposed urban workers. Moreover, when the association between the levels of DNA adducts and the levels of benzo(a)pyrene [B(a)P], a typical marker of environmental air pollution was examined in the studies reporting the measurement of B(a)P concentrations in atmosphere, the dose-response relationship between the levels of DNA adducts and those of B(a)P was found to be linear at low doses and sub-linear at high doses, indicating that the levels of DNA adducts tended to reach a saturation point at increased amount of air pollution. The association between the levels of DNA adducts and those of B(a)P gave also origin to a curve similar to the Michaelis-Menten equation:

$$ V = V_{\text{max}} \times [S]/([S] + K_m) $$

where $V$ is the catalytic velocity of a given enzyme, $S$ is the substrate concentration, and $K_m$ is the Michaelis-Menten constant. The results of this meta-analysis showed that, also in exposed workers, some rate limiting enzyme involved in the activation of PAH type carcinogens may reach a point of saturation at increased concentrations of air pollutants. Deviations from linearity of the dose-response relationship for the formation of DNA adducts are expected when the biologic mechanisms of DNA repair, activation and detoxification of chemical carcinogens are saturated. In chronic exposed workers, the formation of DNA damage may reach a steady-state when the rate of DNA adduct induction is balanced by the rate of DNA adduct removal.

It appears that molecular epidemiology may improve our knowledge of human cancer risk reducing the uncertainties associated with extrapolating from high environmental doses to
low environmental doses. Classical epidemiological study requires relatively high levels of exposure and relatively large samples in order to observe lung cancer relative risks higher greater than 1 at a statistically significant level. The use of molecular parameters may allow for some kind of comparison between an epidemiological significant situation and an epidemiological silent situation. For example, occupational studies have been used to measure a lung cancer unit risk factor of $1 \times 10^{-3}$ (10 percent of people) following a lifetime exposure to B(a)P of 1,000 ng/m$^3$ (Hemminki and Pershagen (1994)). This unit risk factor was used to analyse the annual number of cancers, mainly lung cancers, attributable to urban air pollution, assuming a linear dose-response relationship between higher and lower B(a)P exposure levels (Hemminki and Pershagen (1994)). A lifelong exposure to 0.7 ng/m$^3$ B(a)P was estimated to cause one lung cancer case per year per $10^6$ inhabitants. From Figure 1, we can deduce that the levels of B(a)P of 1,000 ng/m$^3$ and 0.7 ng/m$^3$ correspond to frequency ratios of approximately 3.69 (2.69 over background) and 1.03 (0.03 over background) respectively.

3. Lung cancer

Another achievement of molecular epidemiology has been its application to cancer epidemiology (Veglia et al. (2003)). A meta-analysis of cancer and DNA adducts in molecular epidemiology studies (Veglia et al. (2003)) showed that the levels of DNA adducts may be predictive of lung cancer risk, especially in current smokers. However, the interpretation of this study is limited by the fact that, in case-control studies, the levels of DNA adducts may be related to the cancer disease rather than to the cancer aetiology. Recently, the ability of DNA adducts to predict lung cancer have been investigated in a case-control study nested in the EPIC investigation (Peluso et al. (2005)). This study was performed to evaluate the effects of air pollution the on cancers of the lung, bladder, pharynx, and larynx in never smokers and former smokers in nine European countries, i.e. Denmark, France, Germany, Greece, Netherlands, Italy, Spain, Sweden and United Kingdom. This study was performed using a nested case-cohort design in the EPIC investigation by the means of different biomarkers of exposure, including DNA adducts. The main advantage of this molecular epidemiology study was that the formation of DNA adducts was measured in the blood samples that were collected several years before the onset of cancer. In this manner, the levels of adducts were not influenced by any early effects of cancer itself. Cases included newly diagnosed lung cancer, n = 115, upper respiratory cancers, pharynx, larynx, n = 82, bladder cancer, n = 124, leukemia, n = 166, and COPD or emphysema deaths, n = 77, accrued after a median follow-up of 7 years among the EPIC former smokers and never smokers. Two controls per case were matched for laboratory analyses. Matching criteria were gender, age, smoking status, country of recruitment, and follow-up time.

This EPIC study showed that the levels of DNA adducts were associated with the subsequent risk of lung cancer, with an odds ratio of 1.86, 95% CI 0.88-3.93, when comparing detectable versus non-detectable DNA damage (Table 2). The association with lung cancer was stronger in never smokers, OR = 4.04; 1.06-15.42, and among the younger age groups. After exclusion of the cancers occurring in the first 36 months of follow-up, the OR was 4.16, CI 1.24-13.88.

This study confirmed that the measurement of DNA adducts may identify persons with an enhanced response to carcinogen exposures, certain subjects are inherently more sus-
ceptible to DNA adduct formation and cancer from environmental carcinogen exposures, possibly due to increased metabolic activation of carcinogens, decreased detoxification, and/or decreased DNA repair (Shields et al. (1993), Ryberg et al. (1997), Butkiewicz et al. (2000), Rojas et al. (2000), Teixeira et al. (2002), Palli et al. (2004)).

4. Diet and genetic susceptibilities

Molecular epidemiology has also shown that the levels of DNA adducts reflect primarily environmental exposures to carcinogens, but its production may be regulated from inherited and acquired susceptibilities, including diet and the genetic polymorphisms in metabolic and DNA repair genes (F.P. (2000) Perera et al. (2002)). For example, in an Italian cohort of the European Prospective Investigation into Cancer and nutrition (EPIC) study, the combined effects of different SNPs and selected dietary micronutrients on the formation of DNA adducts have been investigated (Palli et al. (2003)). In particular, the association between DNA adducts and the plasma concentrations of six carotenoids, retinol and α-tocopherol and γ-tocopherol, mainly derived from the consumption of fresh fruit and vegetables, has been analyzed taking into account the genetic deficiency in the detoxifying enzyme glutathione S-transferase M1 (GSTM1 null genotype).

Table I shows that an inverse association between the formation of DNA adducts and the plasma levels of selected micronutrients was present in those subjects with the GSTM1 null genotype. Indeed, DNA adducts tended to be significantly lower in subjects classified in the highest tertiles of plasma concentrations of α-carotene, β-carotene and retinol among volunteers with GSTM1 null genotype. Borderline negative associations also emerged with plasma levels of α-tocopherol and γ-tocopherol. Instead, these associations were not evident among subjects with a GSTM1 wild genotype.

This study shows that some micronutrients contained in the traditional "Mediterranean diet" may inhibit the induction of DNA damage among individuals who do not have the genetic capacity to detoxify carcinogens via the GSTM1 pathway. An increased intake of some common dietary micronutrients of plant origin appears to be sufficient to supply to a genetic detoxifying deficiency, possibly, by interfering with the metabolic pathways of carcinogen activation, or by inducing the detoxification or DNA repair pathways or by antioxidant activity (Mooney et al. (1997), Malaveille et al. (1996); Peluso et al. (2000); Palli et al. (2004)). This is in keeping with previous reports, showing that some dietary components, possibly flavonoids, polyphenols and other natural compounds contained in fresh fruit, vegetables and other typical products of the "Mediterranean diet", may inhibit the formation of DNA adducts, especially in susceptible individuals.

5. Conclusion

Molecular epidemiology has evolved and matured substantially in the last years, providing evidence that environmental chemicals may pose human carcinogenic risks, helping establish the causal roles of environmental factors in cancer, identifying environment susceptibility interactions and groups at higher cancer risk, and giving the possibility of developing new intervention strategies. The predictive value of DNA adducts for cancer risk has been extensively studied, leading
to several human dosimetry studies which have reported associations of increased formation of DNA adduct levels with the occurrence of cancers, including lung cancer (Veglia et al. (2003)). In addition, the formation of DNA adducts has been linked with mutations in the tumor suppressor gene P53 and with early events of carcinogenesis (Pfeifer et al. (2000)).

Molecular epidemiology is now an important cancer prevention discipline that identifies populations at cancer risk and potential interventions to reduce this risk, which are important in generating hypotheses for controlled clinical trials. The recent literature considers DNA adducts as a useful indicator of "DNA damage background" in the whole organism with potential for modification by dietary intervention, with possible relevant consequences for public health.

Diet that emphasize whole grain foods, legumes, vegetables and fresh fruit and that limit animal fat and salt intake have been associated with a decreased risk of a number of diseases, including cancer (Krauss et al. (2000), Byers et al. (2002), WHO (2002); Key et al. (2002)). For example, the Seventh Day Adventists in Los Angeles County consume more natural foods than the rest of Californians and are often vegetarian and abstainers, and although they are living in a polluted area, they show a lower incidence in overall cancer mortality (WHO (2002)). The "Mediterranean diet", characterized by high consumption of plant origin foods, relatively low intake of red meat and high consumption of olive oil, has been associated to a lower cancer incidence, including cancer of colon, breast, bladder and prostate, and a lower risk of cardiovascular disease (Trichopoulou A. (2000)).

Although some dietary patterns have emerged as important preventive factors, much remains to be known about the cellular and molecular effects of dietary constituents, alone or in combination, in decreasing the risk of human diseases, especially with respect to cancer. For instance, studies are on-going to clarify the effect of specific lipids on genes and to understand why ω-3 polyunsaturated fatty acids (PUFAs) inhibit cell proliferation and tumor growth and influence cytokine and Cox-2 mRNA expression (Priante et al. (2002); Vecchini et al. (2004); Aktas and Halperin (2004)).

Scientific interest is now increasing in the study of the effects of individual genetic variation combined to environmental exposure on response to diet and chemoprevention. A nutritional agent may not significantly affect the general population as a whole but only certain susceptible subgroups or individuals. Understanding the individual responsiveness to dietary components may allow to design personalised dietary advice for genetic subsets specifically targeted.

For example, apolipoprotein E and cytochrome P450 7A1 polymorphisms have been associated with change in plasma lipid levels in response to dietary change, including ω-3 PUFAs (Ordovas (2002); Minihane et al. (2000); Hubacek et al. (2003)). The common polymorphism in the methylenetetrahydrofolate reductase gene (677C→T, ala→val) has been shown to affect diet responsiveness of plasma homocysteine (Silaste et al. (2001), a risk factor for cardiovascular diseases (Wald et al. (2002))). Diet responsiveness of paraoxonase-1, an enzyme involved in the development of atherosclerosis, has been shown to be genetically regulated (Rantala et al. (2002)).

The effects of increased dietary intake of polyphenols and other compounds contained in fresh fruit, vegetables and other plants are going to be extensively examined for their capability to inhibit the production of DNA adducts (Malaveille et al. (1996), Peluso unpublished results). Diet rich in vegetables and fresh fruit has been found to prevent the formation of DNA adducts in subjects without the genetic capacity to detoxify carcinogens via the GSTM1 pathway (Palli et al. (2004)).
The ability of endogenous malondialdehyde (MDA)-DNA adducts, a marker of oxidative stress and lipid peroxidation (Munnia et al. (2004), Munnia unpublished results), to induce frameshift mutations in sequences that are models for genetic instability is emerging as a direct link between oxidative stress and a large portion of human cancers. Considering the existing potential for an improvement of the ‘oxidant status’ by dietary preventive interventions - for example, by increasing the dietary intake of lycopene, selenium or green tea (Sharma and Farmer (2004)) - the benefit of incorporating the measurements of endogenous DNA damage in clinical settings is currently examined (Sharma and Farmer (2004), Munnia unpublished results).

The rapid development of high throughput analytical tools and the availability of human genomic databases promises to transform every field of biology, including molecular epidemiology. However, many other important prevention areas need additional development, including trial designs, statistical models to assess risk and other gene specific endpoint biomarkers. Additional resources and more infrastructures linking scientists, policy makers, and other constituencies are needed to increase cancer prevention strategies, also considering that the need for effective cancer prevention will rapidly grow based on predictions that growth and aging of the Western Countries population will double the cancer burden in the next 50 years.

References


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### Table 1: Adjusted geometric means\(^a\) of DNA adducts per \(10^{10}\) normal nucleotides according to tertiles of plasma levels of carotenoids and other micronutrients by glutathione S-transferase M1 (GSTM1) genotype

<table>
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<th>GSTM1 wild genotype</th>
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<td>DNA adduct levels</td>
<td>p for trend</td>
<td>DNA adduct levels</td>
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<td>III</td>
<td>I</td>
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<td>18</td>
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<td>(\gamma)-tocopherol</td>
<td>45</td>
<td>29</td>
<td>24</td>
<td>0.08</td>
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</tbody>
</table>

\(^a\)From a covariance analysis including terms for age, sex, centre, period of blood drawing, total caloric intake, year, body mass index, and smoking history.

### Table 2: DNA adducts and lung cancer in the Gen-Air study. Crude estimates and conditional logistic regression models. In addition to matching variables, estimates are adjusted for education (years of school), BMI, physical activity, intake of fruit, vegetables, meat and energy. Ca/co=cases/controls

<table>
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<tr>
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<tr>
<td>Detectable adducts</td>
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<tr>
<td>non-detectable adducts</td>
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<td>Adjusted by centre</td>
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<tr>
<td>Never smokers</td>
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<tr>
<td>ex-smokers</td>
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<td>0.91-52.1</td>
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<tr>
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<td>1.51</td>
<td>0.67-3.44</td>
</tr>
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</table>
Figure 1: Dose response relationship between FR\textsubscript{i}s and external B(a)P concentrations in work environments (as predicted from equation 6). The insert shows an extrapolated dose-response curve at low exposure doses, assuming a linear dose-response relationship in the range between 0 and 4.5 ng/cm B(a)P, the lowest value of our database. FR\textsubscript{i}, Frequency Ratio for i-th study; B(a)P, benzo(a)pyrene.