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European Journal of Cancer 37 (2001) S67–S87

European Journal of Cancer

www.ejconline.com

Environmental causes of human cancers

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Received 6 December 2000; received in revised form 10 May 2001; accepted 27 July 2001

Abstract

Epidemiological studies have clearly shown a causal association between tobacco exposure and various human cancers, hepatitis B and C infection and hepatocellular carcinoma, human papilloma viruses and cervical cancer, and the occupational origin of certain human cancers is well established. The identification of the environmental causes of human cancers has been a long and difficult process. Much remains to be understood about the role of specific components of the diet and the interaction of different risk factors in the aetiology of human cancers. Withstanding the progress made on the understanding of the cancer process and their potential impact in the therapy of cancer, primary prevention remains, in developed and developing countries, the most effective measure to reduce cancer mortality.

Keywords: Causes; Human cancer; Environmental carcinogenesis

1. Introduction

In 1938, in the essay 'The biology of inequalities', J.B.S. Haldane [1] discusses the difficulty in discriminating the role of the environmental factor "*nurture*" and the genetical factor "*nature*" as causes of human inequalities. In the 20th century, at least in industrialised countries, improvements in living conditions, nutrition and the environment, and to a much lesser extent improved therapy, have resulted in a massive reduction of mortality due to infectious and coronary diseases [2–4]. The understanding of 'nurture', that is the underlying environmental factors causing these diseases and the implementation of primary prevention measures, have been the primary determinants of such healthier lifestyles. Similarly, there is substantial evidence, mainly based on epidemiological studies, that environmental factors are the main causes of human cancers. However, prevention of cancer has been a much less spectacular event and has generally been confined to some developed countries.

These epidemiological contributions include studies of migrants who acquire, already in the first generation, a cancer pattern similar to the native populations [5,6]. Tobacco smoking has been shown to be the major single cause of human cancer with evidence showing that cessation or reduction of smoking results in a significant reduction of risk for lung cancer. Stomach cancer mortality and incidence continue to decrease. Seroepidemiological studies have demonstrated that hepatitis B virus (HBV) infection is causally associated with hepatocellular carcinoma [7], that vaccination against HBV protects against chronic HBV infection [8] and has the potential to prevent primary liver cancer [9]. The impact of therapy in the future reduction of cancer mortality is difficult to assess. Although these observations suggest that one may have an optimistic view, estimations of the future cancer trends indicate that this is not the case. Withstanding any possible reduction of cancer risks or improvement of therapy, population growth and ageing will have the major impact on the future cancer burden (see Parkin and colleagues, this issue). Studies on twins have estimated that the overall contributions of inherited genes to susceptibility to major cancers is minor [10].

Chemicals, viruses and physical agents that have been shown to be carcinogenic in experimental animals are present in the human environment [11] and for that reason have been studied extensively over the past 50 years to examine how they are influencing human cancer rates. The degree of interaction among different cancer risk factors has been examined to a limited extent: this paper reviews the evidence.

We use the term 'environmental causes' with reference to different types of human exposures that include exogenous risk factors (chemical, physical and biological agents) and endogenous risk factors (e.g. oxidative DNA damage resulting from infections, gastric reflux in adenocarcinoma of the oesophagus, etc.). The evidence

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0959-8049/01/$ - see front matter
PII: S0959-8049(01)00266-0

of causality of the association between these environmental risk factors and human cancer is not discussed in detail and the reader is referred to the appropriate International Agency for Research on Cancer (IARC) Monographs (http://www.iarc.fr/). Previous major reviews on the causes of human cancers are those by Doll and Peto [6], Tomatis [12], Cairns [3], Schottenfeld and Fraumeni [13] and Doll [14]. In addition, the paper by Houlston and Peto (this issue of the *European Journal of Cancer*) discusses the impact of cancer susceptibility or insusceptibility to cancer development. Complementary articles are those by Parkin and colleagues and Adami and colleagues (also in this issue).

2. Tobacco smoking and tobacco products

The attempt to obtain a reduction in cigarette consumption has been a long and frustrating undertaking with success being achieved in only a few countries, e.g. USA, UK and Finland. Data from the USA shows that this reduction starts to appear among men in the late 1970s/early 1980s, that is some 50 years after the first evidence of the carcinogenicity of tobacco products (Fig. 1); a similar situation has occurred in some countries in Europe. In the last decade, a decrease in the incidence of lung cancer, associated with a reduced tobacco consumption has been observed in young men [15]. Tobacco companies have exploited all means to undermine the evidence of the carcinogenicity of tobacco products [16]. A historical perspective by Doll [17] describes, in detail, the evidence leading to the causal association between tobacco smoking and various diseases in humans, including lung cancer, and the struggle to implement preventive measures.

In 1931, Roffo reported [18] the induction by tobacco tar of skin tumours in mice and in 1939 Mûller [19] presented the first epidemiological evidence that tobacco smoking is related to lung cancer in humans. Subsequent, large case–control studies [20,21] showed a close association between tobacco smoking and lung cancer risk. Subsequently, prospective studies, with up to 40 years of follow-up, have clearly confirmed these observations and have shown that tobacco smoking is causally associated with the induction of other cancers as well, namely mouth, oesophagus, pharynx, larynx, pancreas, kidney, renal pelvis and bladder, although with lower relative risks. These studies have also shown that regular smoking is associated with an increased mortality due to ischaemic heart disease, respiratory heart disease, aortic aneurysm, peripheral vascular disease and chronic obstructive lung disease [22–24]. Based on these later studies, it was estimated that the cause of death in half of all regular smokers would be due to tobacco use, a much greater risk than previously estimated. In 1995, some three million annual deaths occurred due to tobacco use and it is estimated that by 2025 this figure will rise to some 10 million. This increase will be mainly due to the expected increased consumption of cigarettes in developing countries [25,26].

Some general conclusions can be drawn from these epidemiological studies. Firstly, that in developed countries approximately ~90% of male lung cancer deaths/year and 75–80% of female lung cancer annual deaths/year are attributable to tobacco smoking. The incidence of lung cancer in males in 1990 was 3–4 times higher than in females, which is related to a later start in this century of this habit among females [3]. The incidence of lung cancer in women is still on the increase in most developed countries (see Parkin, this issue).

Secondly, the lifetime relative risk of lung cancer deaths is related to the duration and consumption of cigarette smoking (Fig. 2) [27] reaching an attributable risk of 95% in heavy and long-term smokers [22].

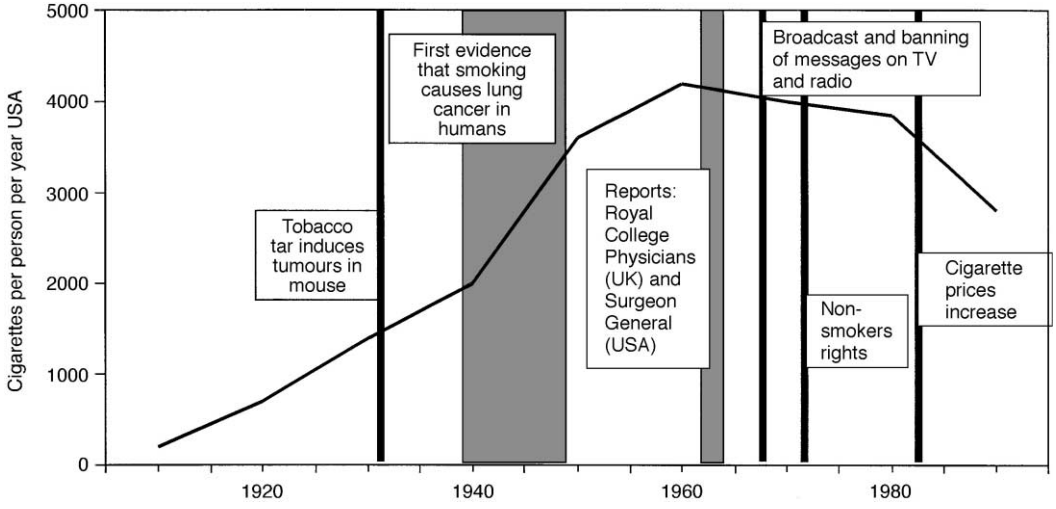

Fig. 1. Cigarette consumption in the USA: the influence of epidemiological and experimental carcinogenicity evidence and public health interventions. Adapted from Refs. [18–21,200].

Thirdly, the risk of lung cancer at age 60 years depends on the age when the individual started cigarette smoking. Adolescents, starting regular smoking at age 15 or less, have a 3–4 times higher risk of lung cancer at age 60 years than a young man starting at age 25 years [3,6,28]. An inverse relationship between the level of DNA adducts in the lung and age at smoking initiation was observed in former smokers; this was not affected by other smoking variables and genetic markers, supporting the epidemiological observation of an independent risk associated with age at the inception of smoking.

Fourthly, stopping smoking results in a considerable reduction of mortality due to lung cancer, as clearly documented in a recent article by Peto and colleagues [29]. This benefit is seen not only in people who stop smoking at age 30 years, but also in people who stop at age 50 or 60 years (Fig. 3). Peto and colleagues [29] estimated that in the UK, "mortality from tobacco in the first half of the 21st century will be affected much more by the number of adult smokers who stop than by the number of adolescents who start smoking".

Fifthly, tobacco smoking will be a major cause of mortality, due to lung cancer and other tobacco-associated diseases, in the next century. For instance, in China it is estimated that "it will kill ... a total of 100 million during the first 50 years of the next century" [30,31].

An analysis of seven case–control studies shows that smoking of cigars, cigarillos and pipe tobacco results in a lung cancer risk comparable to that of smoking cigarettes [32].

In the last decade, considerable evidence has accumulated showing a causal association between lung cancer and environmental tobacco smoking (also called passive smoking). A meta-analysis of 37 published epidemiological studies, that considers various biases and confounding factors, clearly indicates that there is up to a 24% greater risk of lung cancer among non-smoking women if they live with a smoker, and that this risk is dependent on the number of cigarettes and years of exposure to the smoker [33,34]. A similar analysis indicates that there is a 30% greater risk of ischaemic heart disease from exposure to passive smoking [35]. The detection of tobacco specific carcinogens in the blood or urine of non-smokers [36,37] strongly supports the epidemiological evidence that passive smoking causes lung cancer.

In relation to tobacco-associated cancer risks, there are other observations of potential public health relevance that require confirmation. One of these is the risk of the development of childhood cancers. Various published studies, including two large cohort studies, seem to exclude an association between maternal use of tobacco and the risk of childhood cancers [38,39]. A statistically significant trend was observed between paternal daily consumption of cigarettes and the risk of developing cancer in the offspring [38]. However, later studies have failed to show such a trend and there is a clear need for further molecular epidemiological studies, making use of individual markers of exposure to passive smoking, to resolve this issue [40,41]. There is some

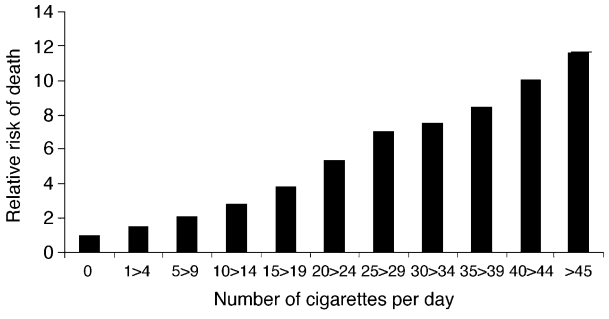

Fig. 2. Lifetime relative risk for mortality from lung cancer according to the number of cigarettes smoked per day [27].

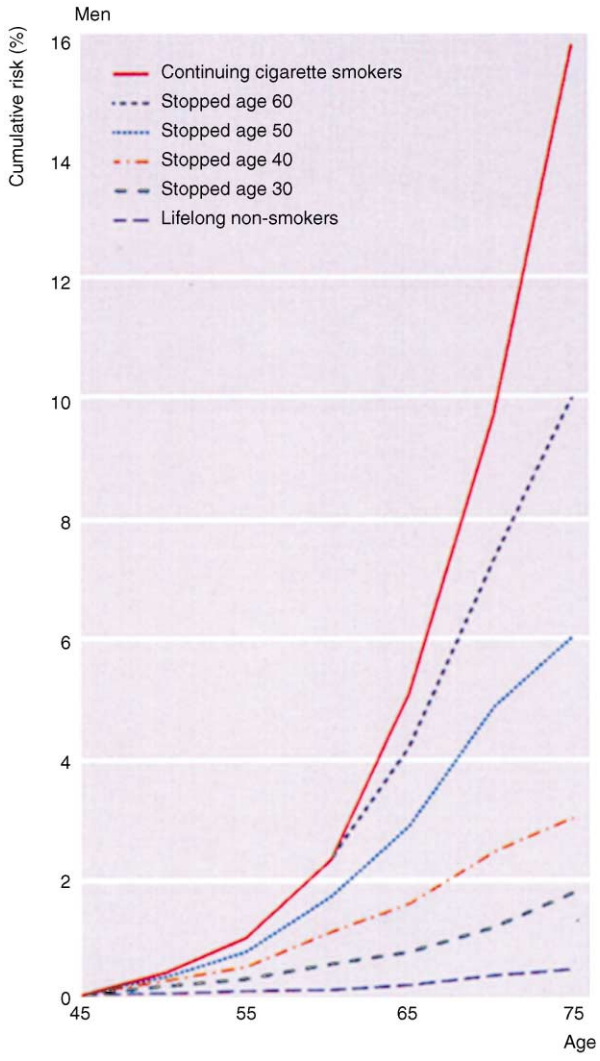

Fig. 3. Effects of stopping smoking at various ages on the cumulative risk of death from lung cancer (up to age 75 years) in the UK [29].

experimental evidence which shows that higher levels of 8-oxo-dG are found in the sperm DNA of smokers compared with non-smokers [42]. It is possible that this could be associated with genetic damage occurring preconceptually in the sperm, although there is no reason to think that this is not as likely to occur in the egg cell, resulting in a similar effect in the offspring.

Some 55 carcinogens, as well as promoting agents, are known to be present in tobacco smoke [22]. It is thus difficult to determine the relative contribution of these different classes of agents to the carcinogenicity of tobacco smoke. Analysis of DNA adducts in human cells and body fluids and experimental carcinogenicity data indicates that polycyclic aromatic hydrocarbons, the tobacco specific nitrosamine 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone and free radicals inducing oxidative damage, are important [43,44]. Other carcinogens, namely 4-aminobiphenyl, nickel, chromium, cadmium and arsenic, are also present in tobacco smoke, but their contribution to the development of lung cancer is less well defined. Some of these carcinogens may have a more important role in other types of cancers associated with tobacco smoking. Many of these carcinogens, as indeed is the case for the majority of the carcinogens, are known to require metabolic activation by specific P450 genes. In the early 1970s, some evidence was provided that the inducibility of the aryl hydrocarbon hydroxylase and certain polymorphisms in other P450s are associated with an increased risk of lung cancer [45]. Molecular epidemiological studies have subsequently examined in some detail the role of carcinogen metabolising enzymes, particularly the cytochrome P450 genes *CYP1A1*, *CYP2D6* and the mu-class glutathione S-transferase (*GSTM1*), as a rate-limiting factor in lung carcinogenesis. The objectives of these studies were to identify if a particular polymorphism in any of these genes, responsible for the activation or detoxification of the different tobacco carcinogens, resulted in a higher cancer risk in smokers compared with non-smokers. Despite the large numbers of studies, the results so far have been on the whole inconclusive [43,46]. The availability of more specific and sensitive methods, such as DNA microarray technology, which are more readily applicable to population studies, may provide more informative data. Large cohort studies [47,48] in monozygotic and dizygotic twin pairs have provided little evidence, if any, of an effect of inherited predisposition on the development of lung cancer and strongly indicate that smoking-induced lung cancer should be attributed to tobacco smoking.

Considerable information is available on the presence and the temporal occurrence in lung cancers of various genetic alterations in oncogenes and tumour suppressor genes and chromosomal abnormalities [49,50]. The alterations, affecting in particular the *TP53* and *p16INK4a* tumour suppressor genes, *K-Ras* oncogene, deletion of the 3p21.3 chromosomal region and amplification of *cyclin D1*, are already found in the premalignant lesions, indicating the relevance of these changes in the process of clonal selection in lung carcinogenesis [51,52]. The spectra of *TP53* mutations in lung cancer is quite distinct between smokers and non-smokers [53,54].

As mentioned above, tobacco smoking is causally associated with cancer at other sites in addition to the lung. It is not yet established whether this range of target organs is due to the action of the different carcinogens present in tobacco smoke or their interaction with other risk factors, or whether there is a variation in the response of the target cells in these different organs to the action of tobacco carcinogens.

Prospective cohort studies, as well as case–control studies, have shown that exposure to tobacco smoking combined with the exposure to some other agents results in a much greater risk of tobacco-associated cancers. This has been shown for oral, laryngeal and oesophageal cancers where the risk of such cancers is higher with the combined exposure of tobacco and alcohol, and for lung cancer with exposure to both tobacco smoke and either asbestos or ionising radiation [22]. For each of these combined exposures the risk of cancer is at least additive and in some cases multiplicative. For instance, in oesophageal cancer in Brittany, France, the relative risk in heavy smokers and heavy drinkers is more that 100 times the risk of non-smokers/non-drinkers with the increased risk associated with alcohol consumption being dose-dependent (see also the next section and Fig. 4).

Tobacco habits other than smoking are also causally associated with cancer in humans. In chewers of betel quid with tobacco, a common habit in South-East Asia, a significant increase of cancer of the oral cavity, pharynx, larynx and oesophagus has been observed [55]. Tobacco chewing and snuff dipping are practised in many parts of the world and are associated with an increased risk of developing oral cancer [55].

3. Dietary constituents/contaminants and nutrition

A detailed comprehensive analysis and evaluation of the evidence that food and nutrition are associated with human cancers has been recently published [56]. Many other informative publications, including the IARC Monographs [57] and recent reviews by Sugimura [58] and Potter [59] have also addressed this issue. There are three dietary constituents/contaminants (alcoholic beverages, aflatoxins and salted foods) that are clearly causally associated with human cancer based on both epidemiological and experimental evidence. The cancer risk associated with over-nutrition leading to excess weight and obesity, and a lack of intake of fruits and

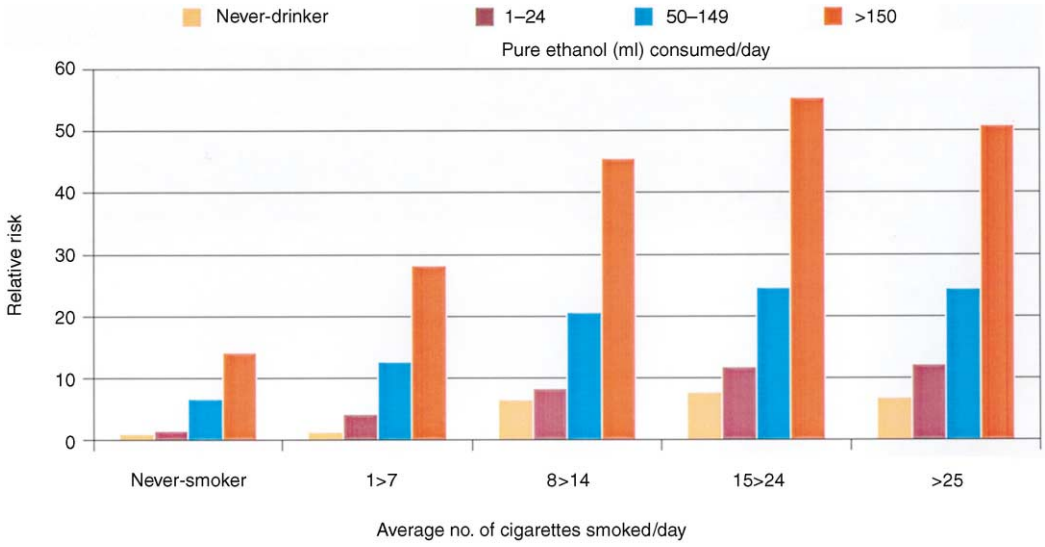

Fig. 4. Joint and independent effects of cigarette smoking and ethanol consumption on the development of oesophageal cancer [67].

vegetables, is equally sound for certain cancer sites (Table 1).

Numerous epidemiological studies have shown that the consumption of *alcoholic beverages* is clearly causally associated with cancers of the oral cavity, larynx, pharynx, oesophagus and liver. This increased cancer risk is still present after adjustment for tobacco smoking, which is also a risk factor for these sites, and there is no sound evidence that this effect is dependent on the type of alcoholic beverage consumed [60]. Epidemiological studies have shown that in addition to alcohol consumption and tobacco smoking, poor nutrition is an important risk factor for the development of oesophageal cancer [61]. The relative contribution of these three major risk factors in the aetiology of oesophageal cancer varies in different regions of the world: nutritional deficiencies are or have been more relevant in certain regions of China, Iran or South-East Africa, whereas tobacco and alcohol consumption are more relevant in other countries (e.g. North and South America and Europe) [62]. It should be noted that in urbanised areas of China, such as Shanghai and Hong Kong, tobacco smoking and alcohol drinking are the major risk factors for oesophageal squamous cell carcinoma as in other developed regions of the world, and that a diet rich in fruit, vegetable and meat products reduces the incidence of this cancer [63–65].

The observations of Tuyns and colleagues [66] of a higher increased risk of oesophageal cancer in those individuals in Brittany, France, who both drink alcohol and smoke has been confirmed and extended in a pooled analysis of five case–control studies, which includes some 2500 subjects from South America [67] (Fig. 4). This latter study confirms that alcohol and tobacco consumption alone were strongly associated with the risk of oesophageal cancer, even in the absence of the other exposures, and shows that the strongest predictors of oesophageal cancer risk is the amount of alcohol consumption per day with a strong dose–response relationship, the lifetime duration of cigarette smoking, and the time since quitting either habit. In addition, the synergistic effect is also seen, in both males and females, among moderate alcohol drinkers and moderate tobacco smokers. Similar findings have been reported for cancers of the oral cavity, pharynx and larynx in a case–control study carried out in Brazil [68].

The important role of the nutritional status (specific nutritional deficiencies/poor nutrition) as an independent risk factor for oesophageal cancer and its interaction with alcohol consumption has been shown by studies carried out in various countries [61,62,69,70].

Experimental carcinogenicity studies in rodents have not provided evidence that ethanol or alcoholic beverages *per se* are carcinogenic, but ethanol has been

Table 1
Diet and dietary constituents/contaminants and human cancers[a]

• Alcoholic beverages	Upper digestive tract and hepatocellular carcinoma
• Aflatoxin	Hepatocellular carcinoma
• Salted fish (Chinese style)	Nasopharyngeal cancer
• Salted and salt preserved food	Gastric cancer
• Over-nutrition leading to excess weight and obesity	Endometrium, kidney, gall-bladder (colon, breast (post-menopausal) and prostate)

[a] Adapted from Refs. [14,56,57,99,100,202]

shown to enhance the carcinogenicity of certain known carcinogens [60]. Acetaldehyde, a metabolite of ethanol, has been shown to be carcinogenic in experimental animals [58]. Molecular epidemiological studies [71,72] have shown that individuals with mutant forms of aldehyde dehydrogenase-2 and alcohol dehydrogenase-2, which result in a high blood concentration of acetaldehyde, have a significantly increased risk of oesophageal cancer. Although these findings point to a more direct role of alcohol in the carcinogenesis process, other possibilities have to be considered and the mechanisms of action of alcohol may be different for the various cancers associated with alcohol drinking.

Studies in rodents, as well as in non-human primates [73–76], have shown that the administration of alcohol results in an inhibition of the detoxification of nitrosamines by the liver and results in a greater amount of the nitrosamines reaching extrahepatic tissues, in which they exert their carcinogenic action following metabolic activation. Nitrosamines are the only carcinogens known to specifically induce oesophageal cancer in rats [77] and the oesophageal mucosa has a relatively high level of the P-450 enzymes which are capable of activating these carcinogens [75]. It is of interest to note that in rodents, a protein-deficient diet has the same effect as alcohol in inducing the inhibition of the first-pass hepatic clearance, resulting in a higher exposure of extrahepatic tissues to nitrosamines [74]. Thus, various mechanisms can be envisaged in the involvement of alcohol in the carcinogenic process in the upper digestive tract and its interaction with tobacco smoking.

Numerous epidemiological studies have shown a consistent increased risk of liver cancer associated with heavy intake of alcoholic beverages [56]. However, as pointed out in this report many of these studies have not considered the contribution of other known risk factors for liver cancer, such as hepatitis B or C infection. In the studies that have taken these factors into consideration, it appears that the critical event resulting from heavy drinking and leading to liver cancer, is the development of cirrhosis [78,79]. The limited knowledge on the mechanisms underlying this process indicates that various types of DNA damage are associated with cell injury due to alcohol and that in cirrhotic liver the disruption of the fine balance between cell proliferation, induction or inhibition of apoptosis and changes in the pool target of liver cell populations critical in cancer development, would facilitate the development of a clone of neoplastic transformed cells [80–82]. The fact that administration of alcohol to experimental animals does not result in the development of hepatocellular carcinoma could be associated with the infrequent occurrence of cirrhosis after chronic exposure to alcohol in these species [83]. This may indicate that in humans, cirrhosis alters the pool size of different cell populations that are the target of various carcinogens. Thus, alcohol may act, at least in human liver carcinogenesis, as an indirect carcinogen by inducing a condition, cirrhosis, that favours the action of other agents, more directly responsible for the process of neoplastic transformation.

Various large prospective studies [84,85] indicate that moderate to high levels of alcohol drinking results in an increased risk of breast cancer. There is some evidence that increased risk of breast cancer is linked with the serum level of endogenous oestrogens [86] and that alcohol drinking raises their levels [87]. Acetaldehyde and reactive oxygen species, formed during alcohol metabolism, may also be responsible for various types of genetic alterations [80].

Aflatoxins are produced by *Aspergillus parasiticum* and *flavus* and are present in various types of foods, aflatoxin B1 (AFB1) being the major component. This dietary exposure is particularly relevant in certain regions of South-East Asia and sub-Saharan Africa [57,88–90]. Aflatoxin B1 is a very potent carcinogen, inducing hepatocellular carcinoma in various animal species [57,91]. The epidemiological studies attempting to estimate the cancer risk attributable to AFB1 exposure or to its interaction with HBV infection in the past have been limited by the lack of markers of exposure to AFB1. In recent years, considerable advances have been made in this direction with a number of individual biochemical and molecular markers being developed which are suitable for use at the population level. These include the detection of aflatoxin B1-albumin adduct in sera and the detection of specific mutations, generated as a result of aflatoxin exposure, such as the Ser 249 *TP53* mutation, which can be measured in small quantities of human body fluids as well as in tumours [89,92–94]. Such individual markers of exposure, have been used at the population level in well-designed epidemiological studies, to estimate the level and prevalence of exposure to AFB1 in different regions of the world (see Fig. 5), the multiplicative interaction with HBV infection in the induction of hepatocellular carcinoma [95] (Fig. 6), and have provided some evidence of an independent role of AFB1 in liver cancer development [96]. These recent publications indicate that the public health relevance of exposure to aflatoxins in certain regions of the world has been underestimated.

Salt and salting of food have long been suspected to be associated with human cancer. At present, there is good evidence that Chinese-style salted fish, together with EBV viral infection, is causally associated with nasopharyngeal cancer. There is also some evidence that the consumption of salt and salted food may increase the risk of stomach cancer [56,57].

An extensive literature is available on the association between diet and cancer [14,56,59,97–99]. The main conclusions from these publications are that dietary habits leading to obesity increase the risk for cancer of the endometrium, kidney, gall-bladder, oesophageal

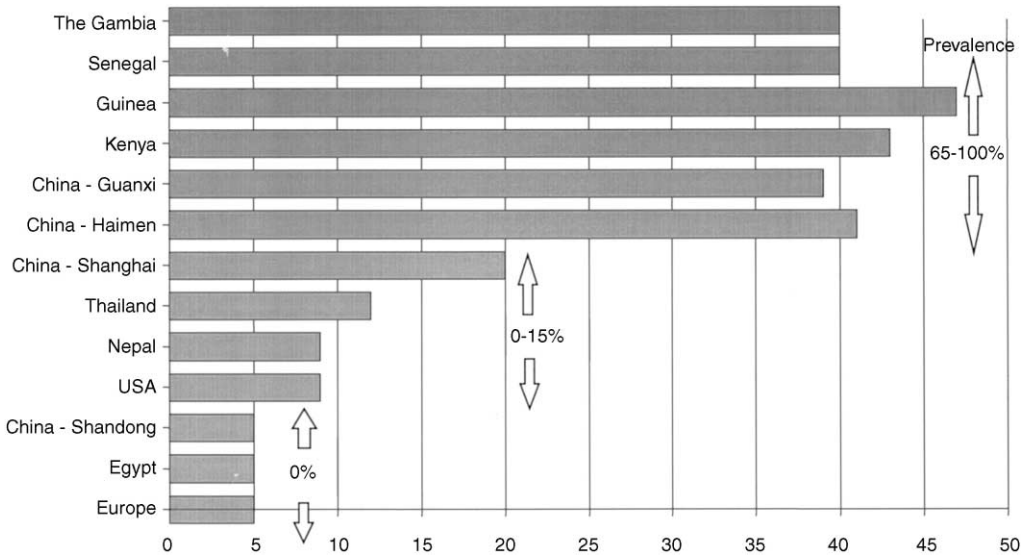

Fig. 5. Level and prevalence of exposure to aflatoxin B1 in populations from various regions of the world [89].

adenocarcinoma and, to a lesser extent, the risk for colon, breast (in postmenopausal women) and prostate cancer. Equally sound evidence shows that a deficiency in the consumption of fruits and vegetables increases the risk of cancer of the upper digestive tract, stomach and lung. A recently-published large prospective cohort study [100,101] reports a null association between the intake of fruits and vegetables and the risk of colon cancer. The role of meat, fat and fibre in the aetiology of cancer in humans is still an open question.

There are other widespread constituents/contaminants of human diet, in particular heterocyclic amines and nitrosamines that are clearly carcinogenic in experimental animals. However, epidemiological studies have so far provided negative or contradictory results in the assessment of the impact of these carcinogens in the aetiology of human cancers. These studies suffer from various limitations, mainly due to the inherent low sensitivity and specificity of dietary exposure assessment in classical epidemiological studies.

Heterocyclic amines are carcinogenic in experimental animals and are present in the human diet, resulting predominantly from pyrolysis products in cooked meats [58]. These carcinogens induce DNA adducts and mutations in various oncogenes and tumour suppressor genes, with interesting similarities between experimental animals and humans [58,102]. However, a recent population-based epidemiological study provided no evidence of an association between cancer of the colon and urinary tract and the intake of heterocyclic amines, as determined by analysis of the consumed food [103].

The risk of cancer development associated with *nitrosamines* was apparent soon after the discovery of this group of carcinogens. These carcinogens are present as such in various types of foods and can also be formed in the stomach from nitrites, which are added to food as preservatives or as a colouring substance to meat, and from nitrosable dialkylamines present in food [77,104]. It was demonstrated that another source of exposure to nitrites resulted from the endogenous formation of nitrates, that are reduced to nitrites by bacteria in the gastro-intestinal tract [105] and also from the nitric oxide synthase (NOS) pathway [106]. Thus, human exposure to carcinogenic nitrosamines is probably more extensive than originally thought, but there is a clear need for more quantitative exposure data using methodologies readily applicable at the population level [58,107]. Epidemiological studies have indicated that dietary exposure to nitrosamines may be associated with cancer of the oesophagus, stomach, infected bladder [108] and more recently with colon cancer [109].

In all these epidemiological studies, either with heterocyclic amines or nitrosamines, it is difficult to draw a

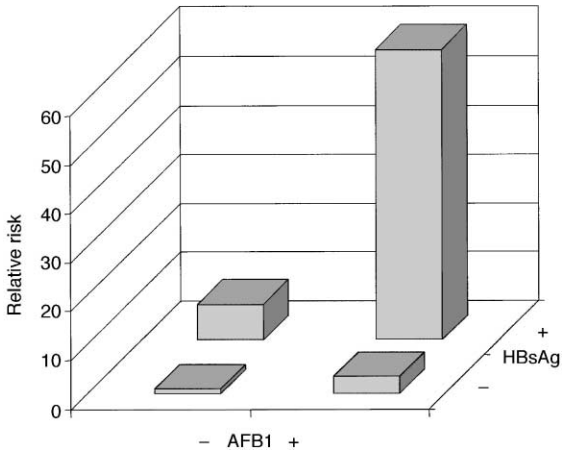

Fig. 6. Relative risk of hepatocellular carcinoma in a population in Shanghai (China) chronically infected with Hepatitis B Virus and exposed to dietary aflatoxin B1 (AFBI) [95]. HBsAg, hepatitis B surface antigen.

sound conclusion of a causal association. The main reason being the difficulty in assessing the dietary exposure with sufficient specificity and sensitivity, and to differentiate the effects attributable to diet as a whole, from those attributable to individual constituents. The integration into epidemiological studies of biochemical, molecular and genetic markers, providing a more reliable measurement of individual exposure to these carcinogens, should overcome some of these difficulties, permitting a better evaluation of the risk of cancer in humans attributable to these carcinogens [14,59,97].

4. Infectious agents and cancer

The first evidence of an association between viruses and cancer was found at the beginning of the last century when experiments in chickens showed that leukaemia [110] and sarcoma [111] can be transmitted by "an agent that passes through a filter". Subsequently, others viruses oncogenic in rodents were discovered, namely mouse mammary tumour virus, murine leukaemia viruses, polyoma virus and papilloma viruses [112].

The first human tumour virus, Epstein–Barr virus (EBV), was identified in Burkitt's lymphoma cells in 1964, and to date several different viruses and infectious agents have been causally associated with human cancers (Table 2). Recent analyses (Parkin and colleagues, this issue) [113] estimate that approximately 1.2 million cancer cases are attributable to infectious agents (approximately 15% of all cancers) and that these cases occur predominately in developing countries. The majority of these cancers have been associated with viruses (10.3%), and the remaining to *Helicobacter pylori* (4.3%) and parasites (0.1%). HBV, human papilloma virus (HPV), hepatitis C virus (HCV) and *H. pylori* account for more than 80% of cancers associated with infections (Table 2).

The estimation of the strength of a causal association between a viral infection and cancer, and the attributable risk differs considerably among the viral infections listed in Table 2, and even for a given viral infection the estimation varies in different regions of the world. The main limitations of these estimations are that:

- They are based mainly on case–control studies; prospective studies, which would overcome the possible influence of the cancer on the serological marker of exposure, are few or not possible to carry out.
- There is a limited availability of informative markers of past exposure to viral infections and/or to other associated cancer risks applicable to epidemiological field studies.
- There is difficulty in assessing the extent and the role of non-viral cancer risk factors in viral-associated cancer.
- There is limited knowledge on the natural history of these cancers and on the cellular/molecular mechanisms of action of infectious agents in the process of neoplastic transformation.

These points are discussed in detail in several recent reviews [112–117].

In the context of the multistage process of carcinogenesis, Weiss [112] describes two general cellular pathways which would lead to malignancy by oncogenic viruses: "*direct oncogenesis*" meaning that the virus infects a progenitor of the clonal tumour cell population, and usually persists in the tumour cells; and "*indirect oncogenesis*" where the virus does not necessarily infect the tumour progenitor cell, but may exert an indirect effect on cell and tissue turnover or on the immune system, which predisposes towards tumour development.

The advances in the understanding of the process of viral carcinogenesis at the cellular and molecular level have been particularly rewarding in the case of human papilloma viruses. These viruses comprise a variety of subtypes with different oncogenic capacity, showing distinct functional properties associated with the process of neoplastic transformation. There is considerable evidence that the oncogenic papilloma viruses alter, by different mechanisms, the function of a variety of proteins involved in various cellular functions (e.g. cell immortalisation, chromosomal stability, mutagenicity and apoptosis), that are critical in the process of neoplastic transformation [118]. Similar functional alterations occur with other oncogenic viruses, namely adenovirus and polyoma virus. Virus infection may also indirectly influence tumour development. In retroviral carcinogenesis, the activation of cellular oncogenes has been demonstrated and during chronic viral infection by HBV or HCV, there may be a selection favouring the proliferation of a target cell population with a tumorigenic phenotype. Impairment of the immune response, as in the case of HIV infection, results in an increased risk of viral cancers [114,119] (Table 2).

It is likely that for a given virus, multiple mechanisms leading to cancer development co-exist. The elucidation of these mechanisms through basic research and the integration of laboratory science into epidemiological studies will allow the causality between exposure to a virus and cancer development to be assessed. This causality has been defined for human papilloma viruses and cervical cancer, as well as human herpes virus and Kaposi's sarcoma [120,121]. Such an approach will be critical in establishing whether there is a causative role in human cancers for certain viruses, namely SV40 [122,123] and JC and BK polyoma viruses [124], as has recently been proposed.

The prospective sero-epidemiological study of Beasly and colleagues in 1981 [7] showed a high incidence of hepatocellular carcinoma among HBV carriers. Since

Table 2
Infectious agents associated with human cancers[a]

Infectious agents	Types of cancer	No. of cancer cases attributable to infections (1990, world total)[b]
Epstein–Barr virus (1964)[c]	Burkitt's lymphoma	6100
	Nasopharyngeal carcinoma	56 200
	Hodgkin's disease	26 200
	Post-transplant lymphomas[d]	
Hepatitis B (1965)	Hepatocellular Carcinoma	228 900
HLTV-1 (1980)	Adult T-cell leukaemia	2600
Human papilloma viruses (1983)	Cancer of the cervix	327 000
	Ano-genital cancer	26 400
	Skin[d]	
	Head and neck[d]	
Human immunodeficiency virus (1983) associated with:		
Human herpes virus-8 (1994)	Kaposi's sarcoma	43 600
Epstein–Barr virus	B-cell lymphoma	8800
Human papilloma viruses (2000)	*In situ* cancer of the cervix, vulva/vagina and penis[d]	
Hepatitis C (1988)	Hepatocellular carcinoma	109 700
Helicobacter pylori (1983)	Gastric cancer	337 800
	Gastric lymphoma	8300
Schistosomes	Bladder cancer	9500
Liver flukes	Cholangiocarcinoma	800
	Total	1 191 900

HTLV, human T cell leukaemia virus.
[a] [112,115,138,203–208].
[b] From Parkin and colleagues[113].
[c] (Year) of identification of the infectious agent associated with cancer.
[d] No data available.

this seminal work, substantial evidence has accumulated that chronic infection due to HBV and more recently to HCV, are the major risk factors for hepatocellular carcinoma in different regions of the world [125]. It has been estimated that these two infections, HBV and HCV, account for ~53 and ~25% of the cases of hepatocellular carcinoma, respectively (see Table 2). There is a better understanding of the natural history of hepatitis B virus infection and of the events taking place between the time of primary infection, which generally occurs during the perinatal period or early childhood period, and the development in adult life of hepatocellular carcinoma, than for HCV-associated liver cancers. However, a number of outstanding questions remain to be elucidated and are discussed in detail in recent publications [126,127]. In brief, they include the reasons for the difference in the rate of transmission from infected (HbeAg-positive) mothers to children in Asia compared with Africa, the elucidation of the route(s) of transmission during childhood of HBV primary infection, the role of nutrition, immunodeficiency (e.g. in HIV patients), immunotoxic agents like aflatoxin, and hormonal status in the establishment and persistence of chronic HBV infection.

A better comprehension of the variables which affect the natural history of HBV infection will have a direct bearing on our understanding of why there is a higher risk in males compared with females of developing hepatocellular carcinoma, the contribution of and interaction with other risk factors, as well as the efficacy of implementing an HBV vaccination programme. It is possible that exposure to AFB1 together with chronic HBV infection is responsible for the high and early occurrence of hepatocellular carcinoma in The Gambia compared with regions like Europe (Fig. 7). Other important risk factors for hepatocellular carcinoma, including exposure to different viruses, may also be associated with this increased risk.

Less information is available on the natural history of HCV infection and hepatocellular carcinoma development [125]. When compared to HBV-associated hepatocellular carcinoma, it is noticeable that in Italy and Japan where over 50% of hepatocellular carcinoma is associated with HCV chronic infection, male predominance is less pronounced and hepatocellular carcinoma occurs in older patients [128]. In certain countries, the prevalence of antibody to HCV in the general population, as in the case of Egypt, is around

20%; this epidemic resulted from iatrogenic transmission during the antischistosomal therapy in the 1970–1980s [129] and an increased incidence of hepatocellular carcinoma is expected to be observed in the years to come.

In the case of HBV infection, various direct and indirect mechanisms associated with liver cancer have been proposed [126,130–132]. These involve alterations of expression and/or mutations of critical cellular genes controlling cell proliferation, cell survival and apoptosis, through different mechanisms, namely insertional mutagenesis, viral trans-activation, alteration of signalling pathways (e.g. Ras-Raf-Map kinase signal cascade), inactivation of the *TP53* tumour suppressor gene and DNA-repair proteins. Considerable experimental evidence indicates that the Hepatitis B virus x-protein is associated with these altered cellular functions.

Another important determinant in the development of hepatocellular carcinoma is the host–immune response to liver cells infected either with hepatitis B or C viruses. Viral replication in infected cells and the concurrent T-cell immune response results in an inflammatory response that persists and manifests itself periodically for many years. In this situation, cell death, fibrosis, leading eventually to cirrhosis, and cell proliferation, entail considerable alterations of the pool size of different liver cell populations. It is reasonable to envisage that in this context there is a higher probability that mutations occur due to oxidative DNA damage or exposure to liver carcinogens like AFB1 [133,134].

In addition, somatic mutations and loss of expression, resulting from epigenetic mechanisms, of several cellular genes, namely the tumour suppressor genes *TP53*, *M6P/IGF2R*, *β-catenin*, *p16INK4a*, and retinoblastoma (*RB*) as well as amplification and increased expression of cellular cyclin D1, have been detected in hepatocellular carcinoma [135–137]. The temporal occurrence of these genetic changes are not well documented, however, it is expected that some will be more relevant to the process of neoplastic development *per se*, and others will be more associated with the aetiology of hepatocellular carcinoma. An example is the high prevalence of a specific *TP53* mutation at codon 249^{ser} detected in hepatocellular carcinoma cases occurring in populations at high exposure to aflatoxin B1, discussed previously, and not in other populations [89].

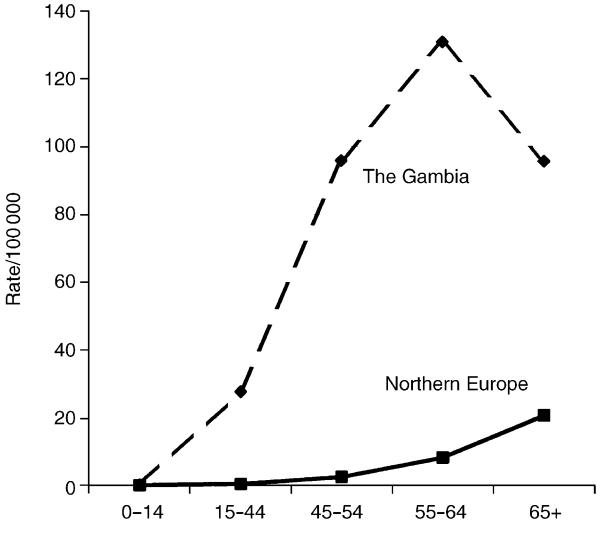

Fig. 7. Age specific incidence rate of primary liver cancer in The Gambia (West Africa) and in Northern Europe [201].

The role of *H. pylori* and of other risk factors in gastric cancer is discussed in the article by Parkin and colleagues in this issue and IARC Monograph, Vol. 61 [138]. Cholangiocarcinoma development, following liver fluke infection and bladder cancer, following infestation with *Scistosoma haematobium*, are discussed in detail in two recent publications [138,139].

5. Radiation

5.1. Ionising radiation (X and gamma rays)

It is now over 70 years since the first report by H.J. Müller in 1927 that ionising radiation (IR) is mutagenic. This was the first demonstration that germ line mutations could be induced by a toxic, exogenous agent. Since then, there have been many studies showing that IR is mutagenic, in both germ and somatic cells, in essentially all experimental systems in which it has been examined (see extensive reviews in UNSCEAR [140–142]. Analysis of germline mutation rates at minisatellites among children born in areas of the Mogilev district of Belarus, which was heavily polluted after the Chernobyl accident, has found that there is a 2-fold higher mutation rate in exposed families compared with non-irradiated families from the UK. Within the Belarus cohort, the mutation rate was significantly higher in families exposed to a higher parental radiation dose, consistent with radiation induction of germline mutations [143,144].

The importance of IR as a human carcinogen has come from studies of two populations. Firstly, individuals who have been exposed to IR and subsequently showed an increased cancer incidence. Observations include carcinoma of the skin of the hand in radiologists, carcinoma of the lung in radon and uranium miners, leukaemia and other cancers in the atomic bomb survivors and among patients irradiated for ankylosing spondylitis [142]. The evidence also shows a linear relationship between dose and cancer risk [145]. The second group of individuals are those with rare cancer-prone syndromes such as Ataxia telangiectasia (AT) and Nijmegen breakage syndrome, in which radiation sensitivity is one of the hall-mark phenotypes. These two distinct, but closely-related diseases are characterised by an extreme sensitivity to IR; homozygotes are

predisposed to developing cancers at a young age, in particular lymphoreticular cancers, and show an acute radiation reaction when treated with conventional radiotherapeutic doses for cancer and heterozygotes have an increased cancer risk [146].

Epidemiological studies have shown that irradiation of the breast especially among young women, increases the risk of subsequently developing breast cancer (reviewed in detail in Ref. [147]). It might thus be expected that genes that are known to influence radiation sensitivity and are associated with an increased breast cancer risk, will be responsible for a proportion of such cases. It is interesting to note that the genes identified to date, belong to a class of proteins that function to maintain genomic integrity. *BRCA1* and *BRCA2* function in DNA repair [148,149], *ATM* both in the detection and signalling of DNA damage and its repair and *TP53* plays a pivotal role in the cellular response to DNA damage. In the absence of any of these proteins, sub-lethal DNA damage would not be effectively repaired and cellular checkpoints for handling such damaged cells would be inactivated leading to an eventual increase in genomic instability.

Occupational radiation exposure to radioactive materials, man-made or natural radiation sources, is incurred by many categories of workers. For the period 1985–1989, it was estimated that approximately four million people worked with man-made uses of radiation [141,142]. In addition, approximately five million workers are estimated to be exposed to natural sources of radiation above background levels. These include coal miners, other underground miners and aircrews. The mortality and cancer incidence of workers employed in the nuclear industry has been assessed in many studies. Based on a combined analysis of workers in the US, UK and Canada, no evidence was found for an association between radiation dose and mortality from all causes or from all cancers. However, mortality from leukaemia was associated with cumulative external radiation dose [150].

In a more recent study [151], the cancer mortality of all the 14 319 workers employed at the Sellafield nuclear reprocessing plant between 1947 and 1975 up until the end of 1992 and the cancer incidence from 1971 to 1986, was examined in relation to their exposures to plutonium and to external radiation. The cancer mortality was 5% lower than that of England and Wales and 3% less than that of Cumbria. A significant excess of deaths from cancer of the pleura and thyroid were found, but for neither site was there a significant association between the risk of cancer and the accumulated radiation dose. Among all radiation workers, there was a significantly positive association between accumulated external radiation dose and mortality from cancers of ill-defined and secondary sites, leukaemia, multiple myeloma, all lymphatic and haematopoietic cancers and all causes of cancer, albeit with different lag times.

The incidence of leukaemia and non-Hodgkin's lymphoma in young people living around the Sellafield plant and certain other nuclear establishments in the UK has been the subject of much debate and research [151]. There have been suggestions of an association between leukaemia in those aged under 25 years and paternal preconceptual exposure to external source of ionising radiation at work [152]. However, one of the most recent studies which has examined this relationship [151] has shown that the overall incidence of cancer and leukaemia among children of nuclear workers was similar to that of the general population. In this same study group, there is evidence of an increased risk of stillbirth with increasing paternal occupational exposure to external radiation which are qualitatively consistent with those from animal models [153].

An alternative explanation for the observed increases of leukaemia recorded near the nuclear reprocessing plants at Dounreay and Sellafield is that they are not due to radiation exposure, but to a virus infection, to which leukaemia is a rare response [154]. These plants were built in unusually isolated places into which large influxes of people occurred, and the study of childhood leukaemia in such populations has given rise to the population-mixing hypotheses. This postulates that the transmission of an infection underlying the cause of the disease is promoted by the mixing of populations with contrasting urban-rural origins and/or socio-economic status [155].

5.2. Radon

Radon is a noble gas that occurs in several isotopic forms, of which radon-222 and radon-220 are found in significant concentrations in the human environment. Radon in the ground, ground-water or building materials enters working and living spaces and disintegrates into its decay products. In comparison with levels in outdoor air, exposure in confined spaces (mines and buildings) is elevated. In many homes in which radon levels are high, the primary source is usually the ground (e.g. granite) on which the house is built. Radon and its decay products have been tested for carcinogenicity in inhalation experiments in some animal species and an increase in respiratory tract tumours were found [156]. In underground miners raised lung cancer rates have been reported from a number of studies. In some studies, there would also appear to be some interaction between radon and its decay products with cigarette smoking with regard to an increased lung cancer risk.

Several case–control studies on lung cancer have suggested a higher risk among individuals with residential radon exposure [157]. Many such studies have been hampered by inaccurate or surrogate measurements for lifetime radon exposure. The specific effects of radon and its decay products have been assessed in relatively

few studies. Its effects are largely attributable to the inhalation of its decay products and the pattern of their deposition in the respiratory tract is dependent on whether they are attached to particles or not.

5.3. UV radiation

Approximately 5% of solar terrestrial radiation is ultraviolet radiation which has differing biological effects depending on its wavelength. Measurable DNA damage is induced in human skin *in vivo* after exposures to UVA (315–400 nm), UVB (280–315 nm) and UVC (100–280 nm) with different photo-products being formed depending on the wavelength. UVA radiation is mutagenic to prokaryotes and eukaryotic cells, induces DNA damage, chromosomal aberrations and sister chromatide exchanges (SCEs) in mammalian cells and DNA damage and mutation in human cells *in vitro*. UVB and UVC have similar biological effects and also induce DNA damage in mammalian skin cells irradiated *in vivo* [158]. Evidence for the involvement of DNA photoproducts in human skin carcinogenesis originally came from the work of Cleaver [159] who showed that cells from patients with xeroderma pigmentosum (XP) are defective in the excision repair of UV-induced pyrimidine dimers from their DNA. XP is a rare cancer prone inherited disorder in which affected patients have more than 1000 times increased frequency of UV induced skin cancer [160]. XP cells are hypersensitive to killing and mutagenesis by UV and since the UV component is the predominant environmental risk for skin cancer [158], a causal association between UV exposure, defective repair of UV-induced photoproducts and non-melanocytic skin cancer has long been postulated. Apart from studies on XP individuals, documentation on the role of DNA repair in the aetiology of human cancers is fairly limited [161–163].

The results of descriptive epidemiology studies suggest that exposure to sunlight increases the risk of non-melanocytic skin cancer and in several cross-sectional studies positive associations have been seen between measures of solar skin damage and the prevalence of basal-cell (BCC) and squamous-cell (SCC) carcinoma. Epidemiological studies have confirmed that exposure to the UV component of sunlight is the major environmental determinant of skin cancer and associated skin conditions and evidence of a causal association between cumulative sun exposure and SCC, solar keratoses and photodamage is relatively straightforward. For BCC and melanoma, this relationship is more complex. Both are associated more strongly with non-occupational exposure than with occupational exposure and the pattern and amount of exposure for each appear to be important [164,165]. Several variables have been identified which are associated with an elevated risk of BCC: having red hair, green, hazel or blue eyes, a tendency to sunburn, and North European ancestry. The lifetime risk of blistering sunburns is also positively associated with BCC risk. Interestingly, living in a region with high solar radiation as an adult was also associated with an increased risk of BCC, whereas living in such a region only in childhood did not constitute a BCC risk. These results confirm the role of constitutional factors and suggest that adult sun exposure increases BCC risk [166]. The efficiency of sunscreens in protecting against skin cancers needs to be carefully assessed. Whilst they do offer protection against UVB wavelengths, their use may increase exposure to UVA and visible light [167]. Both epidemiological surveys and animal experiments have suggested that UVA and perhaps visible light, may induce melanomas [168].

6. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans

The IARC Monographs contain the most authoritative evaluation of the carcinogenicity for humans of various types of exposures, compiled on the basis of epidemiological and experimental data by international groups of experts. These evaluations have a great impact on the implementation of measures of primary cancer prevention at the national and international level and provide the scientific community with a comprehensive source of information for each cancer risk factor. For a complete review of the Monographs, see the publications by Tomatis [11,12,169], who initiated this project at IARC in Lyon, France. At present, 860 types of exposures have been evaluated and 78 of these (Group 1) were considered as carcinogenic to humans (see Table 3a). For the others, the absence or the limited epidemiological data did not permit a more definitive evaluation of their carcinogenicity to humans although many of these exposures were found to be carcinogenic in experimental animals. Group 1 comprises a diversified group of exposures which are of major public health relevance (Table 3b).

More than one third in Group 1 are *occupational exposures* that include asbestos, benzene and aromatic amines. The causal association between asbestos and pleural mesothelioma in the occupational setting has been recognised since 1960 [170] and it has now been shown that environmental exposure to asbestos (e.g. release of fibres from building materials, living close to sites where asbestos is mined and manufactured, or asbestos dust brought home from the workers on the clothes) is also an important route of exposure. A recent study [171] estimates a relative risk of pleural mesothelioma of approximately 8 for household exposure and of 7 for neighbourhood exposure. A projection of future mortality, based on past trends in Western Europe from mesothelioma in males, estimates that a total of approximately a quarter of a million deaths will occur

over the next 35 years [172]. This mortality is mainly the result of occupational exposure to asbestos since the late 1940s. At present, in industrialised countries asbestos exposure has been considerably reduced by stricter control and legislative measures prohibiting the use of some forms of asbestos. The situation is considerably different in other countries (e.g. ex-USSR, China and various developing countries), some of which are the major producers of different types of asbestos and in which the control of exposure is not necessarily implemented [173,174]. Other major occupational exposures to carcinogens in developing countries are to benzene, benzidine, aromatic amines, pesticides, a variety of different metals, and ionising radiation. The transfer of hazardous industries from industrialised to developing countries and the greater number of small factories in such countries in which known carcinogens are still used in the manufacturing processes, such as benzene in the shoe manufacturing industry, will result in a large number of workers at potential risk of developing occupational cancers.

The *medicinal drugs* which are carcinogenic in humans comprise two main groups: namely chemotherapeutic agents and some naturally occurring and synthetic oestrogens and progesterones (see Table 3b). Some of these drugs are very effective in the treatment and prevention of human diseases and the awareness of their possible carcinogenic effect is important when the risk-benefit assessment is made both by the doctor who prescribes their use and by the patient who accepts the treatment.

In 1993, a prospective cohort study [175,176] showed a significant association between fine air pollution particles, derived mainly from the combustion of fossil fuel in transportation, manufacturing and power generation, and mortality from lung cancer and cardiopulmonary disease in six American cities. Results from more recent reports [177–179] are consistent with this observation. In these studies, exposure to tobacco smoking and/or occupational exposure was considered. These findings show that the rise of these diseases due to air pollution, although less important than that induced by tobacco smoking, is present in populations living in polluted cities. In 1989, the IARC classified diesel engine exhaust as Group 2A (probably carcinogenic to humans) [180].

Table 3
(a) Agents evaluated in vols. 1–77 of IARC Monographs on the Evaluation of Carcinogenic Risks to Humans and the classification of their carcinogenicity to humans (http://www.iarc.fr)

Group 1	
Carcinogenic to humans	78
Group 2A	
Probably carcinogenic to humans	63
Group 2B	
Possibly carcinogenic to humans	235
Group 3	
Not classifiable as to its carcinogenicity to humans	483
Group 4	
Probably not carcinogenic to humans	1
Total	860

(b) List of substances and types of exposure classified as carcinogenic to humans (Group 1) in the IARC Monographs vols. 1–77 (http://www.iarc.fr)

• Environmental agents/lifestyle factors[a]	7
• Biological agents[b]	10
• X- and-gamma radiation, neutrons, radon and solar radiation	4
• Industrial processes/occupational exposures[c]	13
• Chemicals or groups of chemicals[d]	23
• Medicinal drugs[e]	21
Total	78

[a] See text. Aflatoxins; alcoholic beverages; betel quid with tobacco; erionite; tobacco products, smokeless; tobacco smoke.

[b] See Table 2.

[c] Aluminium production; manufacture of auramine; boot and shoe manufacture and repair; coal gasification; coke production; furniture and cabinet making; haematite mining (underground) with exposure to radon; iron and steel founding; isopropanol manufacture (strong-acid process); manufacture of magenta; painter (occupational exposure as in footnote a); rubber industry; occupational exposure to strong-inorganic-acid mists containing sulphuric acid.

[d] 4-Aminobiphenyl; arsenic; asbestos; benzene; benzidine; beryllium; bis(chloromethyl)ether and chloromethyl methyl ether; cadmium; chromium [VI] compounds; coal-tar pitches, coal-tars, ethylene oxide; mineral oils; mustard gas; 2-naphthylamine; nickel compounds; shale-oils; silica; soots; talc containing asbestiform fibres, 2,3,7,8-tetrachlorodibenzo-para-dioxin, vinyl chloride, wood dust.

[e] Analgesic mixtures containing phenacetin; azathioprine; busulphan; chlorambucil; cyclosporin; chlornaphazine; cyclophosphamide; diethylstilboestrol; etoposide in combination with cisplatin and bleomycin; melphalan; 8-methoxypsoralen + ultraviolet (UV); MOPP; methyl-CCNU; postmenopausal oestrogen therapy; oestrogens non-steroidal and steroidal; oral contraceptives, combined and sequential; tamoxifen; thiotepa; treosulfan.

7. Molecular epidemiology

Classical epidemiology, through observational, case–control and prospective studies, has provided an enormous contribution to the identification of major risk factors for human cancers. At the same time, epidemiological studies have inherent limitations in the identification of exposure to more discrete widespread risk factors, in the analysis of the complex interaction between environmental exposure and genetic susceptibility of the host, and in the quantification of the relative contribution of different aetiological agents. In the last two decades, new laboratory techniques have been developed that have allowed a better assessment of exposure to carcinogens and other risk factors. The integration of serological markers of viral infection into epidemiological studies was essential in demonstrating the important causal association between viruses and human cancers. The identification of genes critical in the process of carcinogenesis and the development of molecular biology techniques to analyse biological macromolecules from samples easily obtainable in epidemiological field studies, have resulted in a realistic and efficient integration of molecular endpoints relevant to the natural history of cancer into epidemiological studies. Such integrated studies are referred to as '*biochemical, metabolic, hormonal or molecular epidemiology*' [181–185]. Fig. 8 provides a schematic representation of the different approaches in classical and molecular epidemiology. Recent publications [50,89,92,186,187] provide more insight into this area.

The analysis of mutations in genes implicated in tumorigenesis has been one rapidly developing area of molecular epidemiology with many studies being carried out on the tumour suppressor gene *TP53*, which is very frequently mutated in human cancers [188]. The IARC *TP53* mutation database (http://www.iarc.fr/P53/) contains at present some 14 000 mutations. The prevalence, the type of mutations and their temporal occurrence differ considerably among the different tumours that have been analysed. The same tumour type, originating from different regions of the world, can show quite different *TP53* mutation spectra, possibly reflecting their different aetiopathogenesis. Table 4 lists some of the most clear-cut examples of the relationship between a given type of *TP53* mutation and an exogenous carcinogen exposure. Specific mutations (e.g. G to A transition at CpG sites) can also result from altered endogenous cellular processes [189].

The comparison of various genetic alterations, including *TP53* mutations, found in different cancers, can provide valuable insights into their aetiology, pathogenesis and clinical management. Such data for oesophageal and hepatocellular carcinoma are briefly described to illustrate how such markers can be used in molecular epidemiological studies. In both squamous and adenocarcinoma [190] of the oesophagus, there is a high prevalence (up to 80%) of *TP53* mutations. However, the spectra of mutations differ significantly. In squamous cell carcinoma, there is a high prevalence of transitional mutations at A:T base pairs and G to T transversions, whereas in adenocarcinomas G to A transitions at CpG sites are found in up to 60% of samples: this is the highest level of CpG transitions found in any cancer type (see Fig. 9) [191]. The spectrum of *TP53* mutations in oesophageal SCC is consistent with the role of the exogenous carcinogens, tobacco and alcohol, which are the major risk factors identified for this cancer by 'classical' epidemiological studies. A similar pattern of mutations are observed in cancers of the head and neck, the aetiology of which is similar to that of the squamous cell carcinoma of the oesophagus [192]. The presence of transition mutations at CpG sites may be related to alterations in endogenous cellular processes, such as the deamination of 5-methylcytosine. It is not known if this increase in deamination is favoured by gastric reflux, a condition associated with the development of adenocarcinoma. One may also consider that these different patterns of mutation could be related to the different target cells at the origin of these two cancer types.

A specific mutation, a G to T tranversion at codon 249 of the *TP53* gene, leading to an arginine to serine substitution (Ser-249 *TP53* mutation) has been identified as a 'hotspot' in hepatocellular carcinoma occurring in areas where there is a high exposure to AFB1 [193,194]. At present, over 1000 hepatocellular carcinomas from different regions of the world, the majority being from HBV chronic carriers patients, have been examined for *TP53* mutations (exons 5–8) [89]. In regions of high exposure to aflatoxins, such as parts of China and Mozambique, practically all the mutations (40 out of a total of 46) are Ser-249 mutations (Fig. 10). Substantial epidemiological and experimental evidence indicates that this specific mutation is strongly associated with exposure to AFB1. It is also of interest to note that the mutation profile in other regions is completely different, indicating that other risk factors, (e.g. DNA damage attributable to oxidative free radicals, lipid peroxidation and alcohol intake) associated or not with HBV infection, are involved. Recently, the presence of Ser-249 *TP53* mutation was reported in DNA isolated from plasma of individuals from The Gambia with HCC or cirrhosis and exposed to aflatoxins and HBV infection [94]. The measurement of this specific Ser-249 *TP53* mutation, in such an easily obtainable human sample, together with that of aflatoxin–albumin DNA adducts and serological markers of HBV status, may provide a better estimation of the risk associated with these agents and a better understanding of their interactions in cancer development. Such studies will have a direct bearing on assessing the effect of HBV

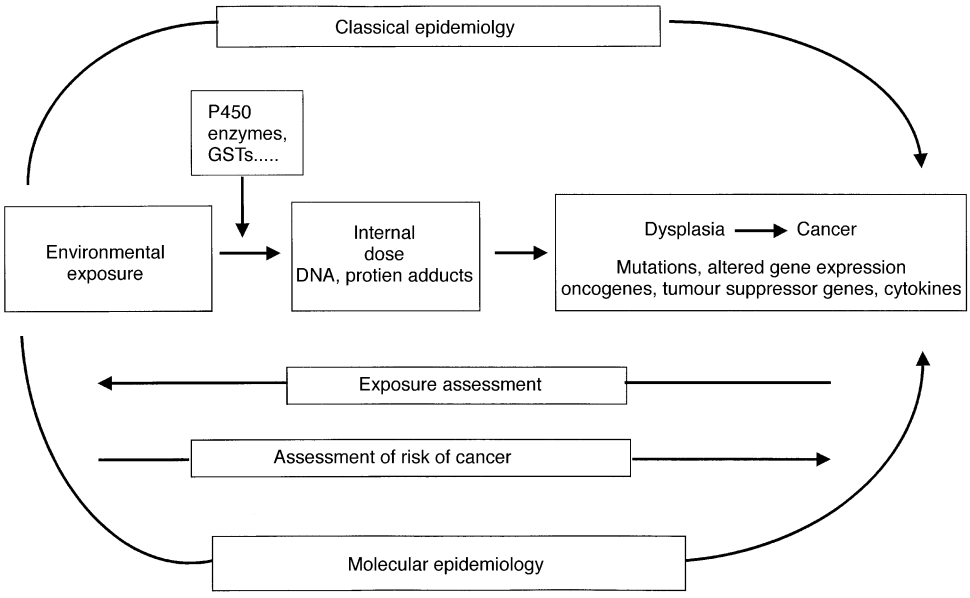

Fig. 8. Schematic representation of classical and molecular epidemiology in studies of cancer aetiology.

vaccination in the prevention of HCC presently underway in The Gambia [8].

The mechanism of *H. pylori* in gastric carcinogenesis is still poorly understood. A recent study [195] shows that the pro-inflammatory genotypes of the interleukin-1 loci increases the probability of the establishment of a chronic hypochlorhydric condition in the corpus of the stomach. This condition favours the development of bacterial infections and thus the production of reactive oxygen and nitrogen oxide species that are mutagenic and carcinogenic.

It is expected that in the near future many more examples of this type will be described, as a result of the availability of various informative polymorphic genetic markers of cancer susceptibility, and individual markers of past exposure to carcinogenic risk factors, as well as techniques suitable for genetic profiling using DNA, RNA or proteins [196]. It is also evident that a better understanding of the natural history of the different human cancers is essential for the design, implementation and analysis of such molecular epidemiological studies.

8. Conclusions

The identification of the environmental causes of human cancers has been a long and difficult process. Epidemiological studies have clearly shown a causal association between tobacco and various human cancers, between HBV and HCV and hepatocellular carcinoma, HPV and cervical cancer, and the occupational origin of some human cancers are well established. However, the aetiology of some of the more common human cancers, including breast, colon, prostate and pancreas, is still elusive and much remains to be understood about the role of specific components of the diet and the interaction of different risk factors in the aetiology of human cancer. It is expected that the integration of laboratory science and epidemiology will provide valuable insights into these unresolved issues. Enormous progress in the understanding of the cancer process has been provided by molecular biology and genetic studies in the last two decades (see Luzzatto and Pontén, this issue), although many unanswered questions remain, in particular the role of gene–environmental

Table 4
TP53 mutations attributable to a carcinogen exposure in a given tumour type

Type of neoplasms	Carcinogen exposure	Mutation type
Hepatocellular carcinoma	Aflatoxin B1	AGG > AGT (codon 249ser)
Skin carcinoma	Sunlight (UVB)	C:C > T:T C > T
Liver angiosarcoma	Vinyl chloride	A:T > T:A
Cigarette smoke	Lung carcinoma	G:C > T:A
Oesophagus/head and neck carcinoma	Tobacco and alcohol	Increased overall frequency of *TP53* mutations

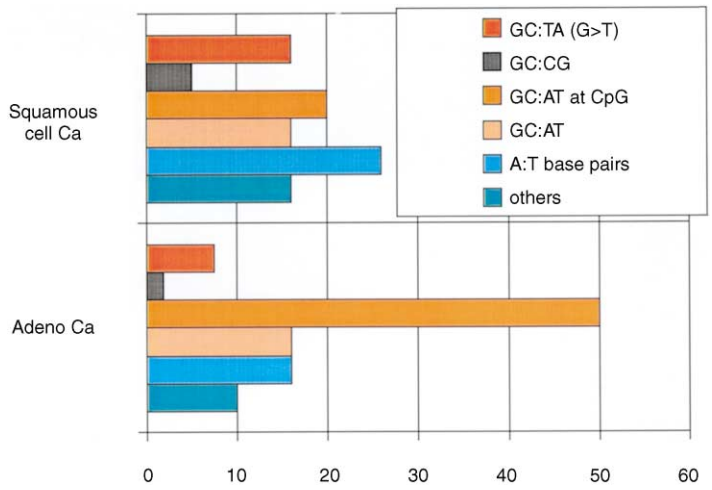

Fig. 9. *TP53* mutation spectra in squamous and adenocarcinoma of the oesophagus (from Ref. [191] and *TP53* mutation database website (http://www.iarc.fr/P53)). Ca, carcinoma.

interactions [197]. While progress in surgery and radiation therapy has contributed to the improving survival of cancer patients, cancer chemotherapy, except for certain types of cancer, has still obtained only limited success [3].

In most developed countries, public health measures have been implemented to reduce exposure to the major identified cancer risk factors, like tobacco, asbestos and occupational carcinogens. These measures have already resulted in the reduction of cancer mortality, as in the case of lung cancer linked to tobacco consumption, discussed above (see also Adami and colleagues, this issue).

The situation in developing countries is, however, quite different and the cancer burden is and will continue to be a major public health problem. The effort to reduce the consumption of cigarettes in these countries will be a long and strenuous task with no assurance of success. In theory, there should be more optimism about reducing the risk of liver cancer associated with HBV infection in these countries. It is proven that HBV infection at birth is causally associated with the occurrence of liver cancer in adolescence or adult life. HBV vaccine has been available since the early 1980s [198]. The introduction of this vaccine in developing countries is relatively inexpensive [199] and its efficacy has been shown against HBV chronic carrier status in the Gambia [8] with promising results in the prevention of pri-

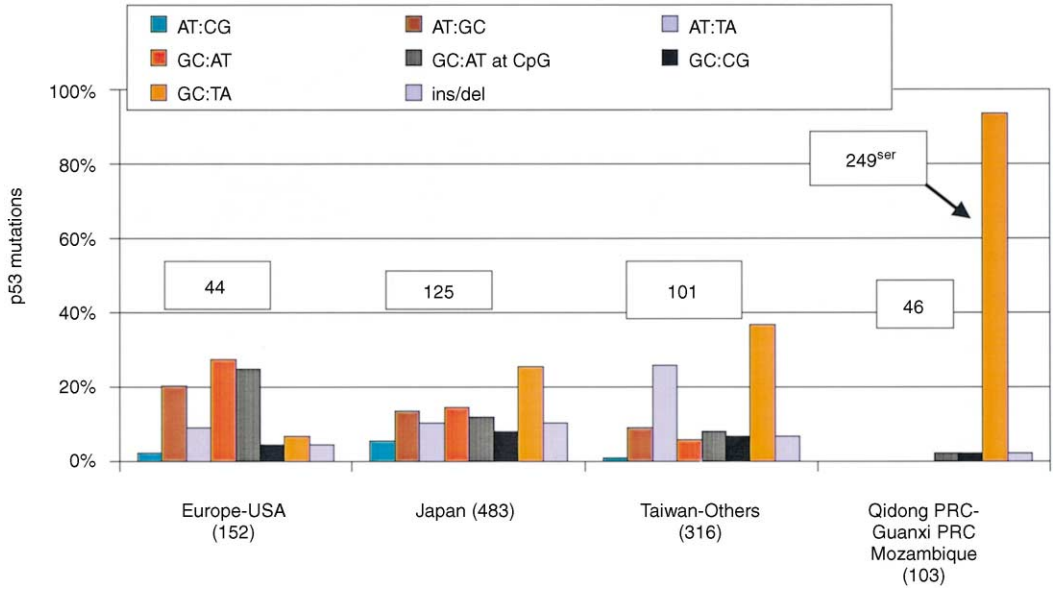

Fig. 10. *TP53* mutation spectra in hepatocellular carcinoma from different regions of the world. The numbers in parentheses refer to the number of hepatocellular carcinomas examined and the numbers in the small boxes refer to the total number of *TP53* mutations detected (exons 5–8) in hepatocellular carcinomas from each region or country. In Qidong, Guanxi and Mozambique, almost all G to T transversions are at codon 249ser. [89] (*TP53* database website: http://www.iarc.fr/P53).

mary liver cancer [9]. HBV is the simplest and most effective intervention known to prevent morbidity and mortality in adults in the world. However, only 50% of children globally and ~1% of those in sub-Sahara Africa have access to the HBV vaccine [199].

Much remains to be accomplished in primary cancer prevention and in the understanding of the natural history of human cancer.

Acknowledgements

The authors wish to thank the following persons for their critical comments and helpful suggestions: P. Boffetta, J. Cairns, J. Fraumeni, J. Goedert, G.D. Kirk, D.M. Parkin, P. Pisani, J. Smith and L. Tomatis; and M. Wrisez for administrative support.

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