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# Mechanisms of Carcinogenesis and Molecular Epidemiology

CARCINOGENESIS IN humans (and animals) is a long process involving multiple changes in genotype and phenotype. The complexity of this process is suggested by several sources of data. The exponential increase in cancer incidence with age can be interpreted as a result of multiple rate-limiting steps in the carcinogenic process, although the number of steps and the biological nature of each step cannot be inferred from the age-incidence pattern alone [1, 2]. Now classical experiments showed that the various steps in the carcinogenic process may occur as a result of the action of different chemical compounds, and that certain exposures may act specifically on cells which have already undergone one or several changes [3]. In colorectal cancer, many of the different steps in the carcinogenic process have been characterised at the molecular level [4]. Each step in the development of cancer may take years to complete and, accordingly, the occurrence of cancer is an event of extremely low probability per cell generation.

Cancer is characterised by unlimited proliferation of cells which fail to respond to physiological control mechanisms, thus destroying surrounding normal tissue, spreading to distant organs and, ultimately, killing the host. The nature of the individual steps in carcinogenesis and the mechanisms that trigger them off are understood only incompletely. New insights into the phenotypic changes required to produce a given cancer are provided by molecular studies of the genetic basis of cancer. Cancer-causing agents or their metabolites may interact with cellular macromolecules to form altered gene products, and the alterations in gene expression may, in some cases, lead to continuous cell proliferation and development of cancer (for references, see [5]).

Research generated during recent years has produced data which suggest that the cascade of molecular events, including chromosomal abnormalities, mutations in cellular oncogenes and tumour suppressor genes, and disturbances in signal transduction and the control of gene expression [6, 7] all play an important role. Disturbances in any of these components could, in theory, lead to uncontrolled cell proliferation and ultimately cancer.

The mechanisms of action of many human carcinogens include both genetic and epigenetic processes [8]. Mechanisms may be understood at many different levels, e.g. for genotoxic carcinogens: metabolism, DNA damage, DNA repair, mutational events, amino acid changes in a proto-oncogene or tumour suppressor gene, changes in the function of the protein, the effect of the altered protein on cellular function or the stage in the carcinogenic process at which the change may be effective [5]. A carcinogenic agent can thus have a multitude of actions which are not mutually exclusive.

In this issue of *The European Journal of Cancer*, Vineis and Brandt-Rauf (pp. 1344–1347) discuss how rapidly evolving knowledge in the molecular biology of the process of carcinogenesis could be integrated into epidemiological studies of human cancer. Epidemiology is the study of the occurrence and distribution of disease in populations, and the identification and quantification of associations between exposures and occurrence of disease. The use of biological measurements in epidemiological studies is an attractive option for the assessment of exposure or for the definition of the outcome under investigation.

For example, specification of the outcome by the presence or absence of a particular mutation in the cancerous cells may yield a higher statistical power for detection of an association, and an improved precision of a quantitative assessment of the relationship between exposure and disease than an analysis of the all-inclusive and often heterogeneous disease entity. The improved specificity may reveal previously obscured 'diluted' or 'averaged' effects. With the emergence of molecular techniques, one may go back to previously conducted case–control studies, apply the molecular techniques to stored samples of biological material from the cases and re-examine the data with separate analysis of each genetically defined subtype of disease.

As an example of the use of biological markers as a means of

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assessing the exposure of cases and controls, a recent study of risk factors for primary liver cancer assessed the exposure to aflatoxins and chronic hepatitis B virus infection by measurement of the levels of aflatoxin $B_1$, its metabolites, and its adducts with DNA, and the presence of hepatitis B surface antigen in previously collected blood samples from 22 cases of primary liver cancer and 140 matched controls from a cohort of 18 244 men in Shanghai [9]. Despite the limited numbers, both chronic hepatitis B virus infection and aflatoxin exposure were found to contribute to the risk for primary liver cancer.

Further potential for application of biological techniques in epidemiology may consist of the study of risk factors for biological effects which are intermediate steps in the formation of cancer, e.g. specific gene mutations in the relevant tissue.

Vineis and Brandt-Rauf emphasise the importance of adequate study design; mechanistic information should be utilised in the context of appropriate epidemiological study design. Most molecular epidemiological studies carried out so far are of a 'transitional' nature: they are small in size, with poor study design, and the representativeness of the population is questionable. Vineis and Brandt-Rauf quote, as examples, investigations of clinical series of cancer cases in which the cases were subdivided by the presence or absence of a molecular marker, and the resulting subtypes tabulated against an exposure variable of interest.

For instance, in one such study, K-*ras* mutations were found in 14 (29%) of the 48 lung tumour specimens examined; the highest frequency of mutations was observed in adenocarcinoma (12/21 samples, i.e. 57%) [10]. The K-*ras* mutations were associated with heavy, life-time exposure to tobacco smoke which is known to contain polynuclear aromatic hydrocarbons capable of causing the G to T transversions observed in the mutated *ras* gene. Recently, Alexandrov *et al.* [11] revealed the presence of benzo[*a*]pyrene diol-epoxide-guanine adducts in lung samples from smokers. These adducts in the guanine bases of DNA are in accordance with the main type of mutations (G to T transversions) found in the K-*ras* oncogene and p53 tumour suppressor gene [10, 12–14]. However, the mutations are also compatible with reactive oxygen metabolite-induced 8-hydroxydeoxyguanine damage.

According to Perera and Schulte [15], molecular epidemiology aims to prevent disease by using biomarkers to identify risks well before clinical onset. The benefits of using molecular biomarkers in epidemiological research are promising, but we need to be careful that we do not merely shift our attention to those things that we can now measure with great precision, looking for the keys under a street lamp when they were lost in a dark alley! Epidemiology deals with humans and human diseases and it should not be an end in itself; its public health goal is cancer prevention. Without doubt, the methods of molecular biology, when used in the epidemiological context, can lead to better epidemiological studies and to earlier detection of potential risks.

Molecular biological studies are critical in elucidating the mechanisms of carcinogenesis. The progress in our understanding of these mechanisms could play an important part in the identification of carcinogenic risks to humans. The *IARC Monographs* programme has recently changed its procedures to allow better use of mechanistic information, with special emphasis on studies in humans, to influence the evaluation of carcinogenic potential more readily [5]. One of the justifications for the changes in the preamble to the *IARC Monographs* was to provide, for the purposes of prevention, a mechanism for the full use of data other than those on cancer incidence or mortality. Ideally, the use of molecular markers in aetiological research may reveal risks well before the clinical onset of cancer.

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