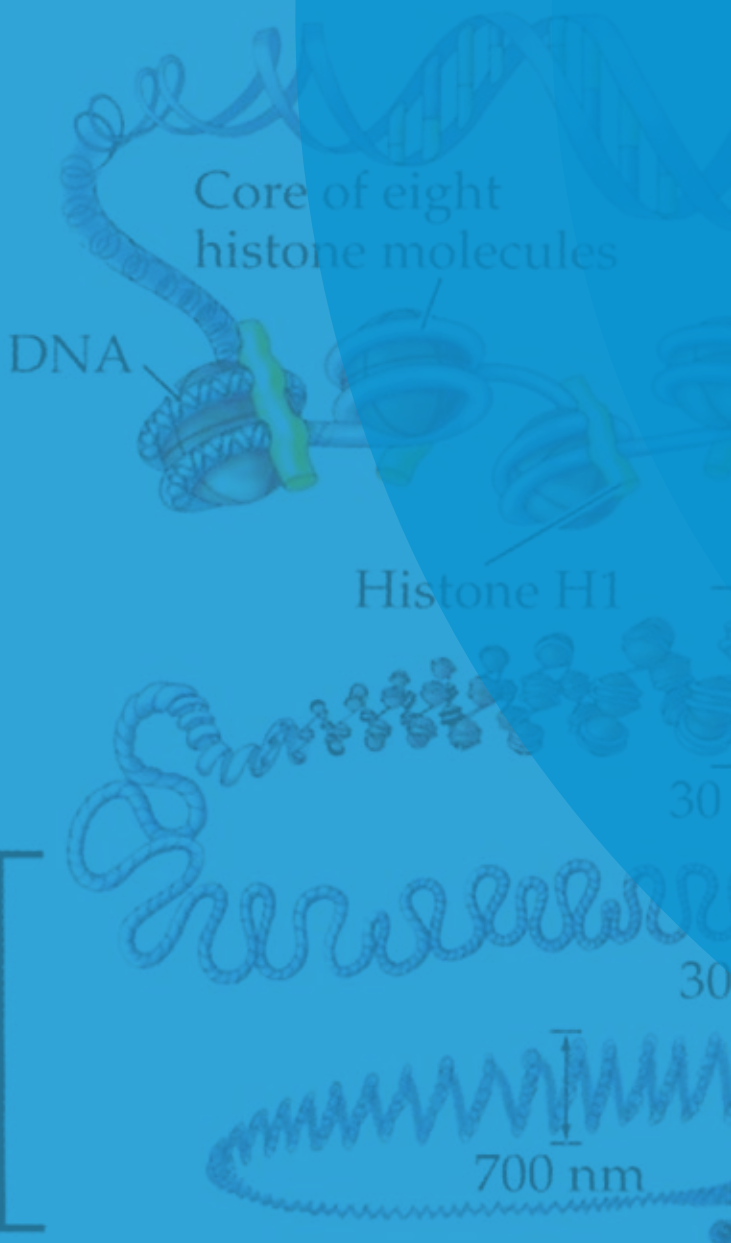

Mutations
Epigenetic modifications
Chromosomal aberrations


Core of eight histone molecules
DNA
Histone H1
30
30
700 nm

FROM LABORATORY TO POPULATION

FROM LABORATORY TO POPULATION

For half a century, IARC has been performing both laboratory-based and epidemiological research. The combination of these two fields, today a common occurrence in cancer research institutions, was infrequent in 1965, when IARC was established. Developing a substantial volume of research in each of these two fields under the same roof keeps scientists abreast of advances in both, helping them to formulate prompt responses to new opportunities for interdisciplinary work. This interdisciplinary approach has given IARC a distinctive profile, not only among cancer research institutions but also within the World Health Organization (WHO), to which it belongs.

The link from laboratory-based research to epidemiology and to public health has been IARC's raison d'être throughout its history. As Helmut Bartsch (whose work is mentioned in the "Biological mechanisms" section of this chapter) pointed out, "In the 1970s, it was realized that an enormous gap existed between laboratory benchwork and studies in humans. IARC researchers played a leading role in a rapprochement between experimentalists and epidemiologists."

> *The raison d'être of the Agency was really to combine epidemiology and laboratory research, and integrate them in order to prevent specific types of cancer.*
> – Ruggero Montesano, former IARC scientist

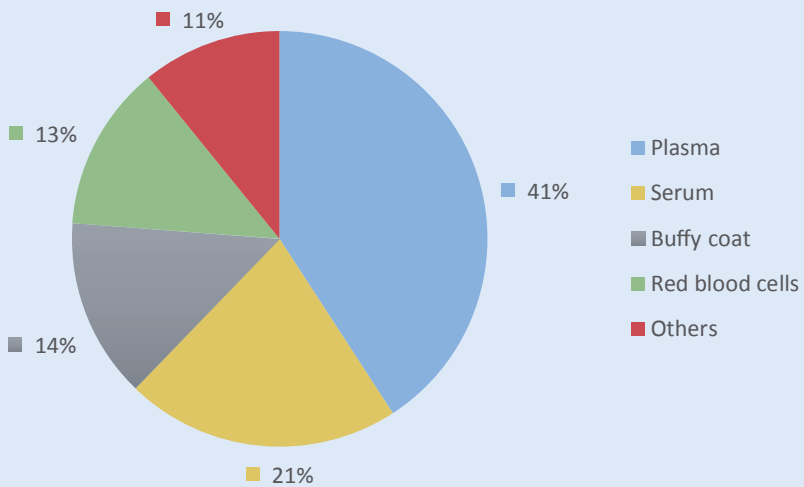
However, this approach also poses challenges. Not all research can be interdisciplinary because each field has its own internal logic and momentum. In a moderately sized institution like IARC (with less than 350 personnel), tensions may arise about the overall direction of research and the allocation of resources. Keeping the right orientation and balancing investments of resources have been constant and major policy concerns for the IARC Directors and the Scientific Council and Governing Council. The approach has been fully justified as radical advances in knowledge and technology in the areas of genetics and epigenetics have increasingly shifted laboratory-based research from studies that are possible only in experimental animals or cell systems to investigations that are directly feasible in humans, on a small or large (epidemiological) scale.

In 50 years, the range of laboratory-based research carried out at IARC has spanned several domains: biological measures (biomarkers) of exposure to agents present in the environment that may cause cancer; genes as potential primary causes of cancer, expanding more recently into epigenetic inheritance; analyses of specific biological mechanisms leading to cancers; and, finally, potential predictors of disease. A large repository of biological samples has facilitated this work, with IARC placing emphasis on samples from epidemiological rather than clinical studies (see "The IARC Biobank").

THE IARC BIOBANK

The IARC Biobank (ibb.iarc.fr) is one of the largest and most varied international collections of biological samples focused on cancer. It contains both population-based collections, from research projects like the European Prospective Investigation into Cancer and Nutrition (EPIC; see the chapter "Nutrition, metabolism, and cancer"), and disease-based collections, which focus on biomarkers, as in the International Head and Neck Cancer Epidemiology (INHANCE) consortium.

The IARC Biobank contains about 5 million biological samples from 1.5 million people. As shown in the figure, most of the samples are body fluids, especially plasma and serum; a substantial proportion consists of extracted DNA. Standard operating procedures are used for accessing, retrieving, and fractioning the specimens and transferring them to laboratories.



The IARC Biobank contains a variety of fluid and tissue specimens. The largest proportions are represented by blood components such as plasma, serum, red blood cells, and the "buffy coat" layer (which contains the white blood cells).

The IARC Biobank is under the responsibility of an IARC scientist and is overseen by the IARC Biobank Steering Committee, in which all research groups are represented. Because one of IARC's major roles is to promote scientific cooperation, a formal policy on access to the samples for research purposes has been developed. As a rule, proposals to access the samples are reviewed for approval by the Biobank Steering Committee and by the IARC Ethics Committee.

IARC also uses its international experience to contribute to the development of best practice in biobanking. A significant landmark was the publication in 2007 of the IARC Working Group Report *Common Minimum Technical Standards and Protocols for Biological Resource Centres Dedicated to Cancer Research*, produced after international consultation. IARC is supporting the adaptation of biobanking best practice to resource-limited settings through the Low- and Middle-Income Countries Biobank and Cohort Building Network (BCNet; bcnet.iarc.fr).

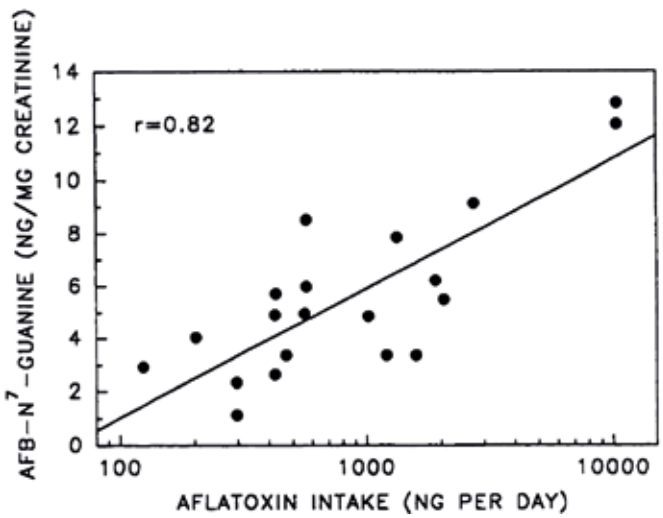
BIOMARKERS OF EXPOSURE TO CARCINOGENS

These biomarkers are indicators of chemical, physical, or biological agents present in the environment that have affected the body. A biomarker may be a chemical that is unaltered and directly measurable, for example when a carcinogenic molecule can be detected in the blood. Or a chemical may be modified in various ways by physiological mechanisms but still be recognizable, for example when the molecule is transformed by oxidation into a specific metabolite. A biomarker may also be the product of an interaction between a chemical and a molecule or cell in the body, for example when adducts (a contraction of "addition products") are formed.

At IARC, an uninterrupted stream of laboratory-based research has focused on biomarkers of exposure – principally the measurement in body fluids or blood cells of carcinogens themselves (free, or bound to some physiological compound) or of the initial damage they may induce in DNA. The need for improved exposure assessment in epidemiological studies is an area where laboratory sciences, along with other technologies, promise significant advances (see "The exposome").

Measuring carcinogenic substances

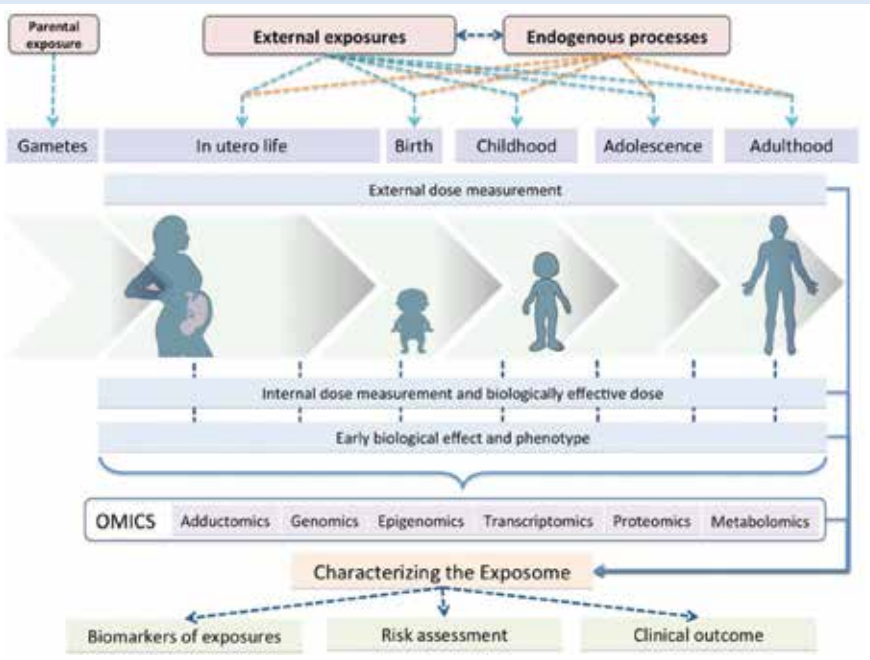
Investigations in the late 1960s and the 1970s on the possible role of aflatoxin ingestion in liver cancer risk in Africa relied on measurements of aflatoxin in food samples (see the chapter "Carcinogens in the human environment"). Much more valid would have been direct measurements of how much aflatoxin a person had actually absorbed from eating contaminated food. The development of pertinent methods of measurement subsequently made it possible to assess aflatoxin levels in body fluids like urine or breast milk. Even better are measurements that enable assessment of the accumulation of aflatoxin as a result of chronic exposure.



Aflatoxin measured in the plate-ready food of 20 Gambians over an 8-day period (horizontal axis) closely correlated with the excretion of an aflatoxin adduct in the urine (vertical axis), thus validating the biomarker as a measure of individual exposure to the carcinogen in the diet.

THE EXPOSOME

The concept of the exposome is currently being implemented and developed as a collaborative endeavour of IARC and scientists around the world. The idea, initially developed by Christopher Wild, is to measure the effects of lifelong environmental exposures on health. It stemmed from the realization that although it is now feasible (and is becoming increasingly cheaper) to explore a person's whole inherited complement of genes, the non-genetic factors potentially involved in cancer causation have been explored only very partially and have been measured one by one. This discrepancy has roots that are biological as well as technical: all genes – unchanged throughout life – can be measured using the same technology (however complex), whereas measuring exposure to highly heterogeneous and time-variable environmental factors requires disparate technical methods. However, recognizing that all these factors belong to an ensemble – the exposome – prompts both the search for common methods of measurement and a better-organized, more systematic approach to the assessment of the great variety of exposome components (depicted in the figure).



The exposome comprises every exposure to which an individual is subjected over a lifetime. Exposures arise from two broad categories: external and internal sources. External exposures include different environmental and lifestyle factors (e.g. chemicals, infectious agents, diet, tobacco, alcohol, and socioeconomic factors). Internal exposures include endogenous processes (e.g. metabolism, hormones, inflammation, and gut microorganisms).

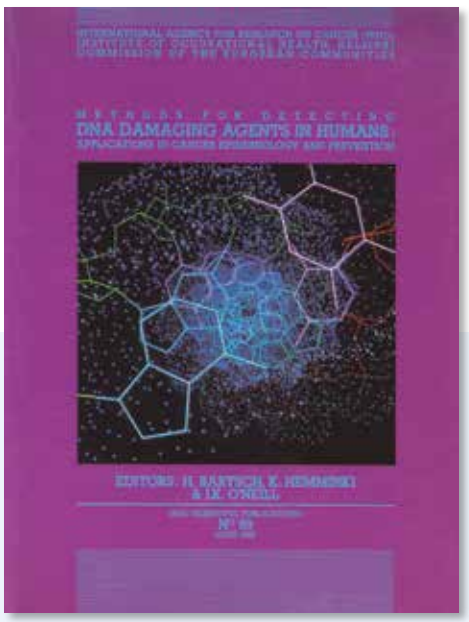
The concept of the exposome may help drive research efforts to improve exposure assessment and to generate new hypotheses about the causes and prevention of human cancer. As outlined in the figure, each person undergoes a multitude of exposures, starting from in utero life, via the mother, and continuing throughout the life-course (in fact, exposures affecting the sperm and ova of parents may also be relevant). Exposures arise from two broad categories: external and internal (endogenous) sources. External exposures include different environmental and lifestyle factors such as chemicals, infectious agents, diet, tobacco, alcohol, and the social determinants of disease. Internal sources include processes such as metabolism, hormones, inflammation, and gut bacteria. The measurable fingerprints of these exposures characterize the exposome. They can be of practical utility to recognize, and then remove, a carcinogenic exposure, or to assess the size of the cancer risk associated with it, or for an early clinical diagnosis of a cancer.

Aflatoxins bind to proteins such as albumin and to DNA to form adducts. Levels of aflatoxin–albumin adducts in blood samples serve as a biomarker for assessing chronic exposure, while aflatoxin–DNA adducts in urine provide a shorter-term measure. Thus, measurement of such adducts has become one of the tools of public health programmes targeted at the detection and removal of this food contaminant.

Laboratory methods for measuring biomarkers of exposure expanded rapidly in the late 1970s and the 1980s, as highlighted in *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention*, a 1988 IARC review publication that included several contributions from IARC laboratories. It became possible to reliably measure potential carcinogens from a variety of sources: diet, polluted air (outdoors or in a workplace), medications, alcoholic beverages, and others. As a result of the enormous interest in this area, from 1978 to 1993 IARC published a series of 12 technical volumes, *Environmental Carcinogens: Methods of Analysis and Exposure Measurement*, describing validated methods for analysing chemicals and mixtures ranging from volatile nitrosamines to indoor air (see "Standards and safety").

Damage to DNA

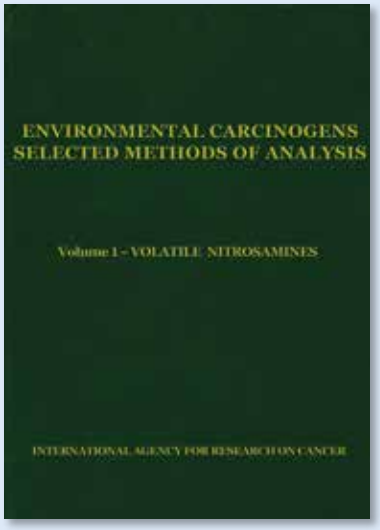
The binding of a carcinogenic molecule to DNA may be only the first step leading to the damage of DNA. The resulting deleterious change in the DNA structure will persist if several defence mechanisms are overcome. The consequences may range from a simple substitution in one of the DNA base pairs to a large rearrangement of a chromosome. Genetic mutations that play roles in cancer development are continually being identified.



STANDARDS AND SAFETY

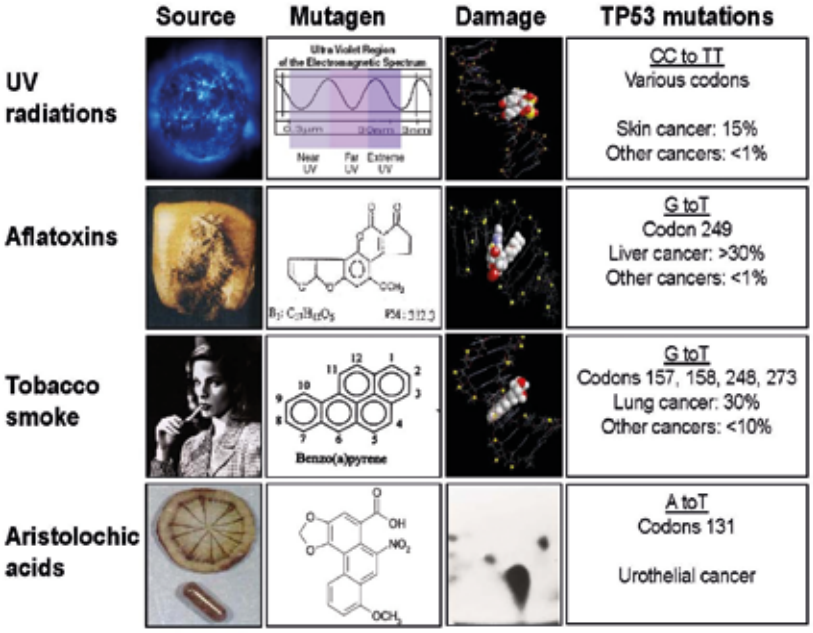
IARC's expertise in laboratory technology and mechanisms of carcinogenesis resulted in valuable resources being made available to the cancer community more widely. In this context, IARC played an important role in reporting on standardized methodologies to measure carcinogens or related biomarkers. For example, the publications on *N*-nitroso compounds, mentioned in this chapter, started with volumes that presented analytical procedures to measure this wide family of carcinogens in various types of samples. This approach was expanded to cover other carcinogens, for example vinyl chloride.

In other instances, IARC supported laboratories in improving their analytical accuracy and precision. A notable example was the mycotoxin check-sample programme, where food samples with known levels of mycotoxins were distributed to laboratories worldwide for analysis. The investigators were able to subsequently compare their results to those of other centres. A similar principle was later followed to measure DNA damage using the ^{32}P-postlabelling technique, where standard DNA specimens were provided by IARC and analysed by participating laboratories.



In addition to supporting method validation for chemical analyses, IARC laboratory scientists at different times provided evaluations of cell transformation assays for short-term testing of carcinogens as well as texts on the pathology of tumours in animals. These publications were most frequently released as volumes of the IARC Scientific Publications series subsequent to international workshops of the leading experts in the field.

As cancer research expanded, the potential risk of exposure of experimental scientists and others handling chemical carcinogens was recognized. IARC therefore developed a series of manuals on *Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes* to provide clear guidance on safe disposal. This series covered many different carcinogens, including polycyclic aromatic hydrocarbons, aflatoxins, hydrazines, aromatic amines, and haloethers.



Four examples of mutation patterns and cancer "fingerprints". The sources portrayed in the first column (ultraviolet radiation, nuts contaminated with aflatoxin, tobacco smoke, and botanical products containing aristolochic acid) contain the carcinogens shown in the second column, which each produce a specific adduct, as depicted in the third column, and a particular TP53 *mutation pattern, described in the fourth column.*

Ruggero Montesano, seen here in his laboratory, joined IARC in 1970. For three decades, he was responsible for developing the field of mechanisms of carcinogenesis into several specific research areas. His constant priority was to focus on ways to connect mechanisms with feasible investigations in large human populations.

TP53 is a tumour suppressor gene that acts via a protein (p53) as a gatekeeper, protecting the integrity of cells against a large group of tumour-promoting processes. Mutations that inactivate *TP53* are an important step on the path to cancer. They are found in all cancer types, with frequencies that vary from 5% to 90%. In some instances these mutations are scattered along the whole DNA sequence, while in others they are concentrated at a few mutation hotspots.

IARC scientists were among the first to carry out research on these *TP53* mutation patterns, recognizing their potential value as fingerprints of past exposure to environmental carcinogens. According to Ruggero Montesano, the key element was the connection between work in the laboratory and at the population level: "There was a lot to do in the laboratory, which was a new one starting from scratch, to develop a technology for measuring mutations, which had to be relatively simple in order to be applicable in thousands of people. ... We were aiming not to discover 'the cure for cancer' but to understand the natural history of cancers through mutations caused by specific and removable factors in the environment, like aflatoxin for liver cancer." The subsequent extension of these analyses to measure a common aflatoxin-associated mutation in codon 249 of the *TP53* gene in the plasma of liver cancer cases provided a promising proof of principle for the

early detection of cancer through non-invasive molecular tests. At IARC, a database was developed by Monica Hollstein that currently documents all *TP53* variations reported in the scientific literature (p53.iarc.fr). More than 30 000 mutations occurring in tumours are included, accompanied by a rich annotation of tumour characteristics.

Fingerprints of exposures in cancer cells

TP53 mutation patterns are just one example of how environmental exposures may be traceable in tumour cells through the scrutiny of complex patterns of genetic changes. IARC scientists recently investigated renal cancer in tumour samples from four countries: the Czech Republic, Romania, the Russian Federation, and the United Kingdom. The analysis of the whole genome showed a striking difference in the frequency of a particular type of mutation between countries. The far higher frequency found in the samples from Romania opens up the possibility that renal cancers in that country may be caused by a specific environmental exposure. A good candidate is aristolochic acid. This chemical, which is contained in some herbal remedies and weight-loss preparations, is a known carcinogen. Aristolochic acid is also the cause of a renal disease prevalent in some areas of the Balkans, and it causes the types of mutations seen in the renal cancer samples from Romania.

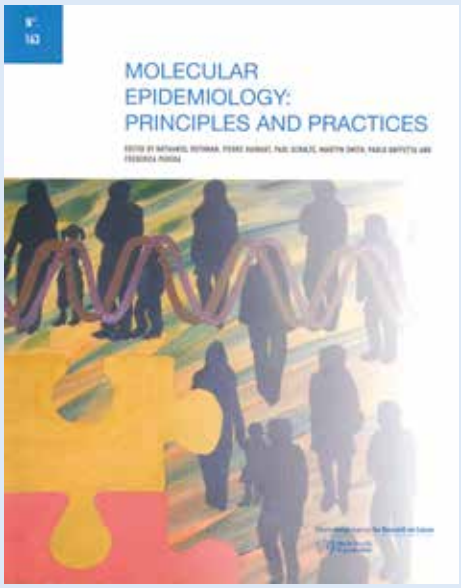
Exposure fingerprints of various types form an important part of the broad field of molecular epidemiology (see “Molecular epidemiology”).

Mutation patterns in tumour samples from patients with clear cell renal cell carcinoma in four countries. The columns in the small graphs indicate the number of A:T > T:A mutations per sample, out of a total number of 20 000. These mutations consist of changes (transversions) of an adenine–thymine base pair (A:T) into a thymine–adenine pair (T:A). Patients from Romania have an unexpectedly high frequency of A:T > T:A transversions, consistent with exposure to aristolochic acid.

MOLECULAR EPIDEMIOLOGY

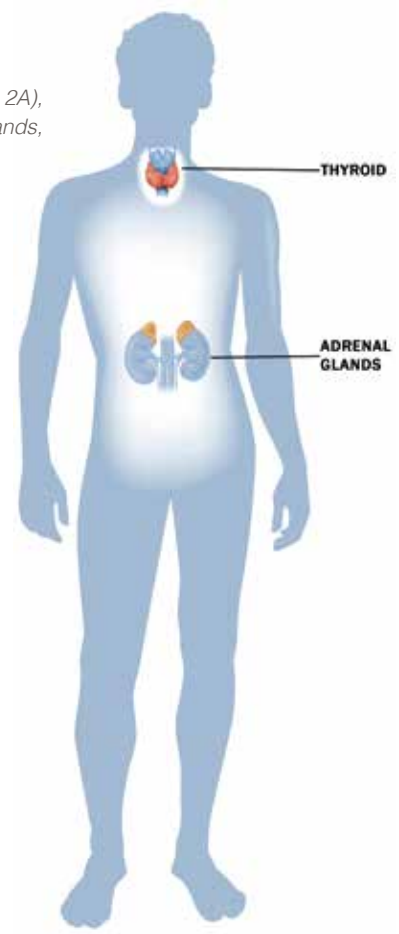
In epidemiological studies, often a substantial portion of the information is gathered through questionnaires, for example enquiring about characteristics such as sex, age, educational level, diet, and tobacco use. There is also a long history in epidemiological research of direct measurements in the human body, particularly since it became possible – in the second half of the 19th century – to isolate disease-causing bacteria from organisms found in animals and humans. Immunological markers of exposure to microorganisms followed, and measurements of blood cholesterol and lipids have been available for decades in cardiovascular epidemiology. However, the blossoming of molecular biology has hugely amplified the scale on which biological traits, ranging from exposure fingerprints to genes and gene products, can be usefully incorporated into epidemiological studies of cancer and other diseases. This increase in scale has necessitated the development of new statistical methods and bioinformatics tools to organize and interpret the vast amount of information generated.

The 2011 IARC publication *Molecular Epidemiology: Principles and Practices* is an extension of work that began 25 years earlier, with IARC's 1986 course on "Molecular biology for epidemiologists", and continued with several IARC courses on molecular epidemiology (see the chapter "Education and training of cancer researchers"). In the style of a methodological textbook, more than 60 scientists from IARC and institutions around the world present a comprehensive survey of the current status of molecular epidemiology – a broad label that embraces epidemiological studies using measurements of any kind of biological molecules, from small ions of, say, sodium or potassium to large structures like DNA or proteins. Molecular epidemiology is a principal instrument of today's translational medical research, centred on converting the results of basic research into tools for clinical practice and public health.



GENES AND CANCER

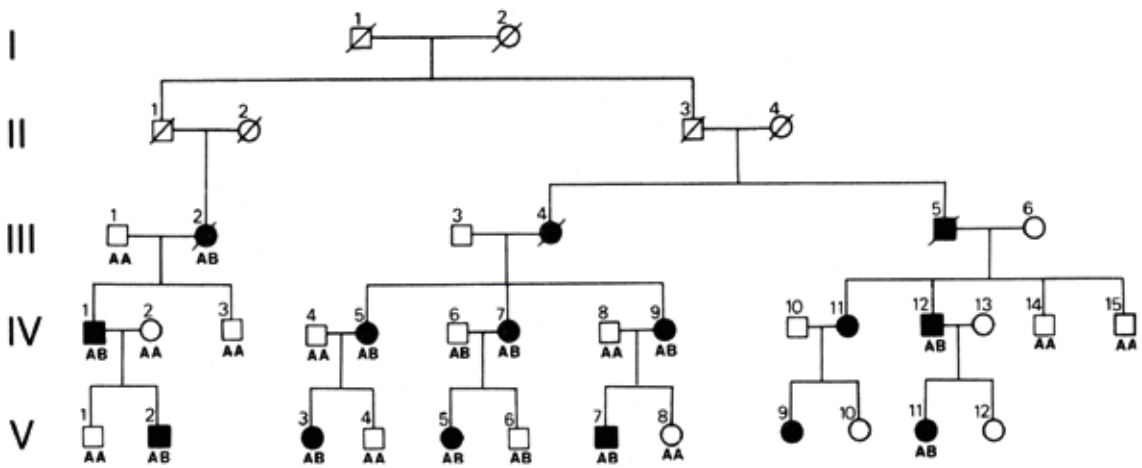
Cancer has been defined as a genetic disease because gene alterations are key steps in the processes that transform a normal cell into a cancer cell capable of propagating to the stage of clinical cancer. However, genes can also predispose to cancer when particular variants occur in the germ cells (sperm and ova) of parents. For example, a rare hereditary mutation of *TP53* is transmitted from parents to offspring as a dominant gene, conferring a very high risk of cancer in one or more of several organs (the breast, soft tissue and bone, the brain, and bone marrow).



In multiple endocrine neoplasia type 2A (MEN 2A), cancers develop in the thyroid and adrenal glands, which are hormone-secreting organs.

Studies of cancer-predisposing genes have been carried out at IARC since the technology first permitted the direct detection of inherited genetic variants. Priority was given to cancers that often occur with elevated frequency within families. The aims were both to better understand the biological basis of the predisposition and to make genetic counselling possible by identifying the individuals at risk within such families. An early example was a condition called multiple endocrine neoplasia type 2A (MEN 2A), which is genetically inherited and affects 1 in 25 000 people, in whom cancers of the thyroid and adrenal glands develop.

To detect and remove such MEN 2A-associated cancers at a very early stage, it is important to regularly screen for neoplastic changes in those relatives of affected people who carry the genetic variant responsible. In the late 1980s, a collaborative IARC study in France identified three DNA markers that enabled the identification from a young age of people carrying the version of the gene that confers a high risk. A similar approach in the USA in five large families with hereditary transmission of predisposition to breast and ovarian cancers resulted in the identification of a region on chromosome 17 where the genetic variant responsible for the predisposition is located.



Example of a multigenerational family with members affected by MEN 2A. Circles indicate females and squares males; filled symbols show affected individuals, and slashes denote those who are deceased. The letters represent the combination of genetic variants in a person tested for a DNA marker in order to provide genetic counselling. For example, subject V-1 was a 15-year-old boy not (yet) affected by the disease. The testing had established that the mutation causing MEN 2A was associated with the B variant in the father. Although the B variant was passed on to subject V-2, subject V-1 had only A variants, and thus he could reasonably be reassured that he would never develop the disease.

DNA analyses of members of families with an unusually high frequency of cancers, notably breast cancer, were fast multiplying. Therefore, in November 1989, IARC convened an international workshop on Linkage Studies of Hereditary Breast Cancer, to critically review the methodological aspects of these studies and scrutinize the validity of the results that had already been acquired. To accelerate the pace of discovery of genes responsible for hereditary breast cancer, a network was launched whereby data submitted by participant scientists would be tabulated, summarized, and redistributed to the contributors. Gilbert Lenoir had promoted the initiative with Bruce Ponder from Cambridge, United Kingdom. Lenoir noted that IARC was in an ideal position to enter the young field of genetic epidemiology, because of the Agency's experience in organizing the large international collaborations needed to successfully assemble an adequate number of families affected by uncommon hereditary cancers throughout the world. More generally, according to Lenoir, IARC's reputation was such that "an IARC business card opened doors and made everything possible, because of the link with WHO. For example, to set up a new collaboration, it was enough to send a letter mentioning WHO/IARC/Lyon and you would always receive a reply, while this would not necessarily happen for a letter emanating from another institution."

At IARC, Gilbert Lenoir (left) combined research on viral carcinogenesis with the initiation and development of the cancer genetics programme. After holding a professorship in medical genetics at the University of Lyon, he became scientific director of the Gustave Roussy Institute in Paris. Here, Lenoir is with Nobel Prize laureate Harald zur Hausen on the occasion of the awarding of the 2009 IARC Medals of Honour to zur Hausen and Nubia Muñoz, for their discovery that human papillomaviruses cause cancer of the uterine cervix.

This strength, arising from the combination of technical capability, extensive experience, and status within WHO, has sustained IARC's expanded role in international genetic epidemiology until the present day. Research in this field has shifted focus: from rare genetic variants entailing a very high risk of cancer, as was found with the uncommon *BRCA1* and *BRCA2* genes responsible for a small fraction of breast cancers, to common genetic variants that each potentially contribute a small increase in risk. Recent IARC-coordinated studies exploring the whole genome (genome-wide association studies, or GWAS) have identified several genetic variants potentially involved in the causation of renal cancer, cancers of the upper respiratory and digestive tracts, and lung cancer. When the evidence from more than 4000 lung cancer cases and 7000 controls in five separate studies (including the European Prospective Investigation into Cancer and Nutrition [EPIC] and the IARC Central Europe lung cancer study) was combined, a genetic variant located on the long arm of chromosome 15 was found to be associated with an increased risk of lung cancer.

EPIGENETICS

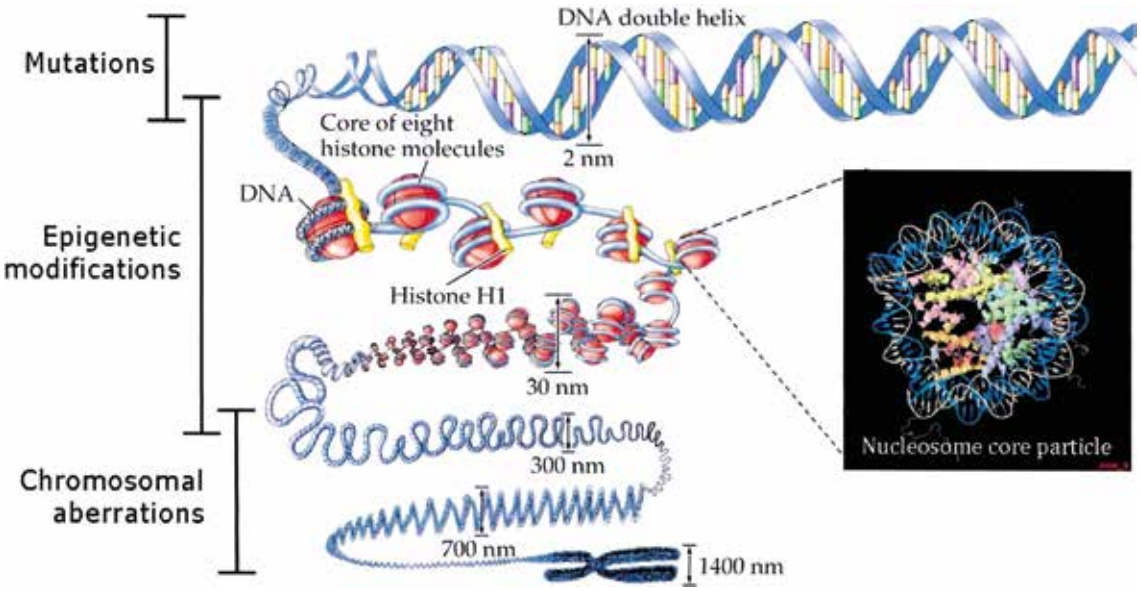
Epigenetics is a new, rapidly expanding field of research in cell biology, including cancer biology. Epigenetics encompasses the study of all changes in gene expression that are passed on from one generation of cells to the next but do not involve changes (such as mutations) in the DNA sequence itself. The emergence of epigenetics has challenged the dogma that the only heritable characteristics are those coded in the DNA sequence. It has also opened up a vast field of research on heritable epigenetic changes that are induced by environmental exposures, presenting novel ways to study the mechanisms by which such exposures lead to cancer development.

As in the case of mutations, laboratories at IARC are developing ways to measure epigenetic alterations in the minute amounts of tumour DNA that can be found circulating in the blood. This makes it realistic to apply these sophisticated measurements to biological samples collected and stored for epidemiological studies. Early initiatives are showing how different diets may result in epigenetic changes involved in the development of breast cancer.

> *In the same building there were people with expertise in epidemiology and in understanding mechanisms, and I enjoyed the cross-talk. I learned from it and I think it has sustained me since I left IARC. There was that wonderful cafeteria and coffee bar on the top floor; I think that is where the real collaboration happened.*
> – Julian Little, former IARC scientist

BIOLOGICAL MECHANISMS

Research on biological mechanisms of cancer development has often focused on elucidating epidemiological findings, in terms of underlying physiology and pathology. This approach of investigating the biological plausibility of an

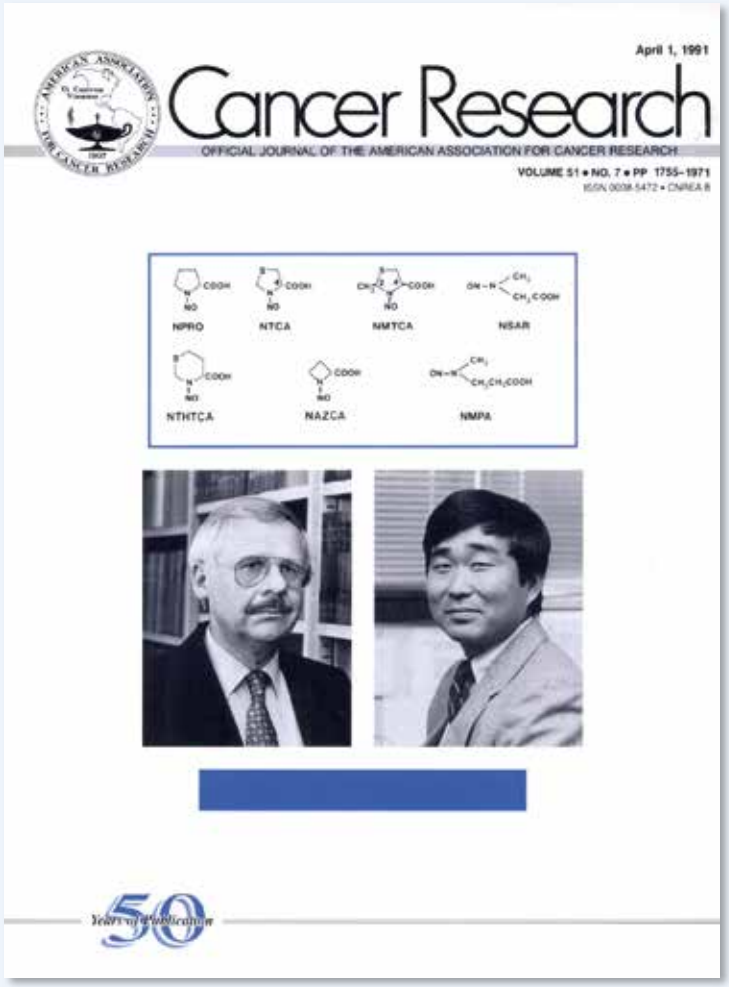


The DNA double helix, which can be modified by mutations, is folded into larger nucleosomes, which are the target of epigenetic changes. The nucleosome chain is in turn folded and packed into the even larger chromosomal structures, which may be altered by gross aberrations. Sizes are indicated in nanometres (nm), billionths of a metre.

epidemiological association has been used for many different agents. For example, *Biological Effects of Asbestos* was one of the first IARC Scientific Publications, and co-editor Pavel Bogovski involved IARC in experiments exploring the relationship between the physical and chemical properties of different types of asbestos fibres and their carcinogenicity. More generally, methods of testing chemicals for mutagenic activity in bacteria were developed as rapid tools for recognizing mutagenic carcinogens. This type of information on mechanisms has supported programmes for the identification of carcinogens in the environment (see the chapter "Carcinogens in the human environment").

For many years IARC placed great emphasis on *N*-nitroso compounds. These compounds had been known since the 1950s to be potent carcinogens in a wide variety of experimental animals. However, a host of unanswered questions remained about their measurement, distribution, and sources in the human environment, and their actual role in human cancers. *N*-nitroso compounds were fascinating partly because of the wide range of organs affected by different members of this family of chemicals in rodent studies. To keep abreast of progress, IARC organized an international conference in 1969 and published the proceedings in its Scientific Publications series; over the next two decades, 10 further volumes on *N*-nitroso compounds followed, at two-year intervals.

In parallel, IARC laboratories tackled the emerging issue of endogenous formation: *N*-nitroso compounds are not only found preformed (often in minute amounts) in the environment, such as in some foods, tobacco smoke, and polluted air, but – more importantly – are also formed within the body from the precursor molecules nitrate and nitrite, which are widely present in drinking-water. In 1981, Helmut Bartsch and Hiroshi Ohshima reported on a simple and reliable non-invasive test whereby the amino acid proline was given orally and scavenged nitrosating agents, leading to formation of *N*-nitrosoproline, which could be measured in the urine. This test enabled the exploration of the body's capacity to form endogenous *N*-nitroso compounds, which may contribute more than half of a person's total exposure to these chemicals. The test was applied widely

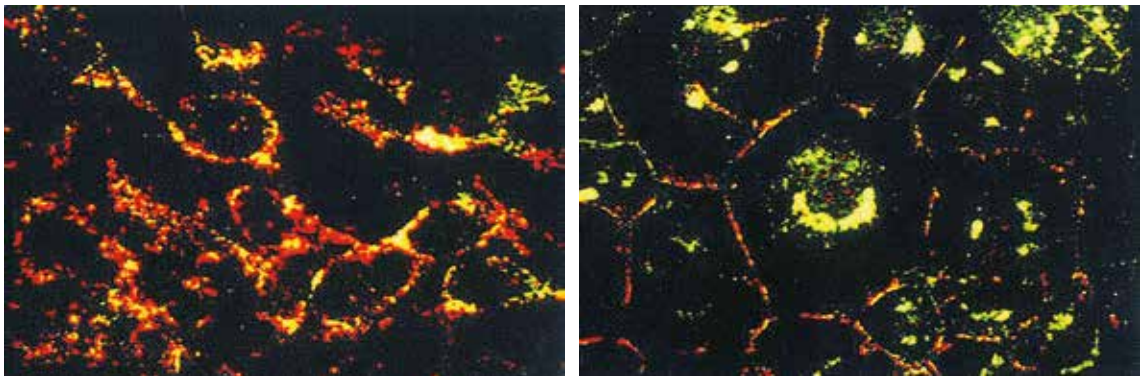


The April 1991 issue of the journal Cancer Research *featured Helmut Bartsch and Hiroshi Ohshima on the cover and highlighted their development of a simple, sensitive, and non-invasive method for the estimation of endogenous nitrosation in humans. Bartsch (left) conducted research at IARC from the early 1970s to the early 1990s. He then became head of the Division of Toxicology and Cancer Risk Factors at the German Cancer Research Center in Heidelberg. Ohshima (right), a graduate of the Tokyo University of Fisheries, joined IARC in 1979 and moved in 2006 to the Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, Japan.*

in epidemiological studies, including an assessment of endogenous nitrosation across 69 counties of China in comparison to oesophageal cancer mortality rates. An understanding of the underlying chemistry also led to the demonstration of a reduction in endogenous nitrosation by ingestion of vitamin C, a potent inhibitor of nitrosation.

In the mid-1980s, IARC scientists were among the first to show that the DNA adducts induced by *N*-nitroso compounds, which had been seen in experimental animals, could also be detected in human tissues. These results came from studies of oesophageal cancer in Linxian County, China. *N*-nitroso compounds also appear to be implicated in stomach cancer, together with the major causative factor, infection with the bacterium *Helicobacter pylori*. This finding comes from earlier IARC laboratory studies and recent results from the EPIC team (see the chapter "Nutrition, metabolism, and cancer"). *N*-nitroso compounds generated from nitrate and nitrite in foods containing red and processed meat may be linked to causation of colorectal cancer, while those formed endogenously by microorganisms that infect humans might play a role in the development of cancers of the bladder and biliary tract.

The adducts formed when *N*-nitroso compounds bind to DNA may, if not repaired, induce mutations that are important in transforming a normal cell into a cancer cell. DNA repair is a defence mechanism in which DNA damage is identified and mended to maintain the integrity of the genetic code. Substantial research into



Gap junction intercellular communication is mediated by molecules called connexins. Under the microscope, two types of connexins appear as fluorescent green and red (left image) within cells in culture. When the fluid medium in which the cells are bathed is changed from a low to a high calcium concentration, the connexins migrate to the cell membranes (right image), altering the cell-to-cell communication capabilities.

DNA repair processes has been conducted in IARC laboratories. Chronic (rather than acute) administration of *N*-nitroso compounds to experimental animals was found to increase DNA repair by excision of damaged DNA, an adaptive defence response of the organism to the carcinogen. The DNA damage response was also investigated in heritable conditions, such as ataxia telangiectasia, in which the repair processes are genetically impaired, thus predisposing the affected people to cancer occurrence.

After the initial damage to DNA and associated mutations, the next step in the process that leads to cancer involves a progressively increasing capacity for disordered growth and proliferation of cells. In normal tissues, cells constantly communicate and interact to coordinate and maintain proper functioning. Regulated communication between cells occurs through gap junctions, specialized structures that allow the passage of chemical messages from one cell to another. Mechanisms that disrupt this cell-to-cell communication were studied extensively by the team of Hiroshi Yamasaki, who was a co-editor of the book *Cell Differentiation*, *Genes and Cancer*, published by IARC in 1988. For Yamasaki, combining basic research with IARC's commitment

For about 20 years, Hiroshi Yamasaki directed IARC's research programme on the mechanisms of intercellular communication that ensure orderly functioning in normal tissues and on how carcinogenic agents can interfere with or disrupt this communication.

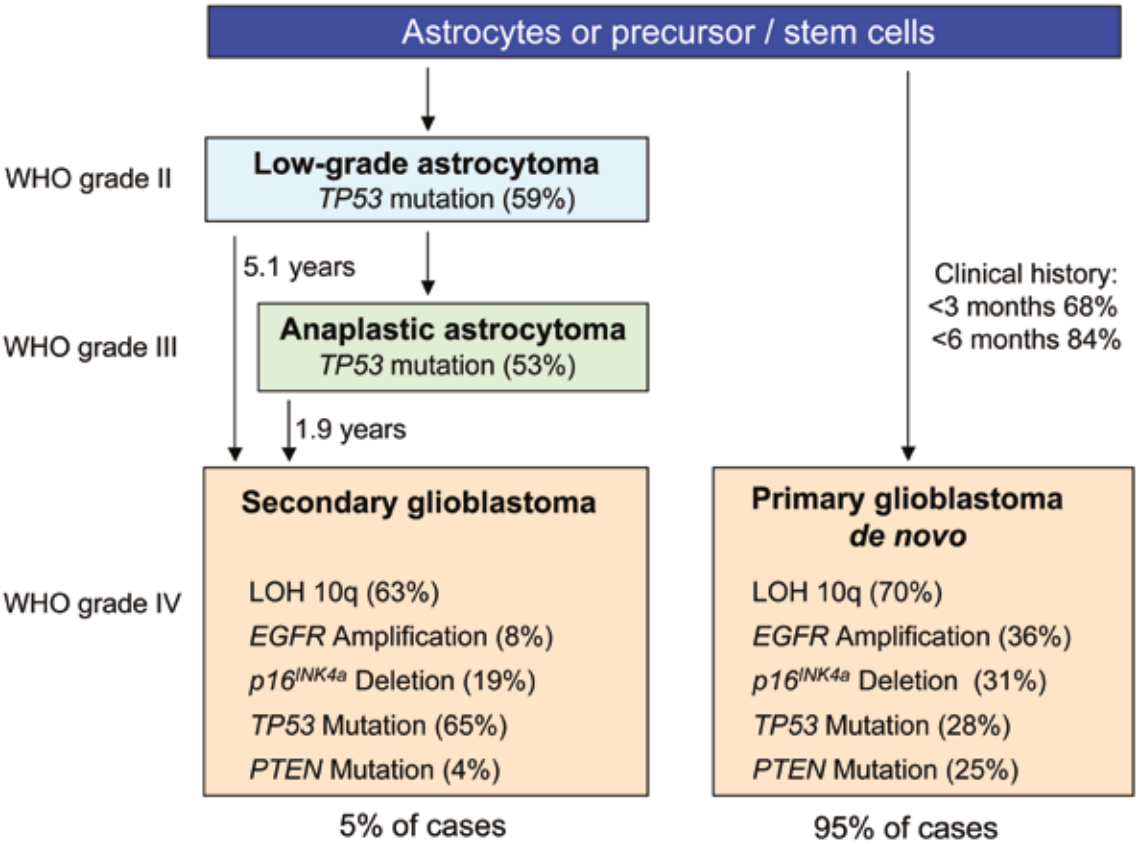
to cancer prevention was a key concern: "The most challenging task was to satisfy my own scientific appetite in basic research and to follow IARC's mission on public health. As a laboratory scientist, it was important for me to keep up with cutting-edge cancer research. At the same time, it was important to consider that IARC is a public health institute and its mission is cancer prevention. It was quite challenging to balance these two elements. I tried to balance them in several ways: first, I obtained much competitive funding for my basic research; second, I used my basic research knowledge to contribute to the IARC Monographs Programme; and third, I applied basic research to molecular cancer epidemiology."

In several body tissues, cell proliferation may also be influenced by hormones. A series of studies have been investigating the role of hormones in cancer development, using the biological specimens from the EPIC study (see the chapter "Nutrition, metabolism, and cancer"). Most of the analytical determinations have been performed in IARC laboratories, which have adapted the assays to the requirements of large-scale epidemiological studies. Studies have been conducted mainly on cancers of the prostate, thyroid, colorectum, ovary, and breast. Clarifying the confusing picture that had emerged from previous studies, it was shown that elevated levels of androgen and estrogen hormones, but not of progesterone, increase the risk of breast cancer. Another study found that in postmenopausal women, higher levels of insulin-like growth factor I, which regulates cell proliferation, may increase the risk of receptor-positive breast cancer, but not of receptor-negative breast cancer, the other principal subtype. As these examples show, laboratory-based research on metabolic and hormonal factors enables the identification of specific paths leading to cancer, which are potentially controllable by pharmacological (chemopreventive) means.

PREDICTORS OF DISEASE

Most biological processes related to cancer can be investigated from two perspectives. The "upstream" approach considers direct or remote evidence of causes of cancer, either genetic or environmental. The "downstream" approach identifies predictive indicators of the likelihood of cancer occurrence, or of the course and outcome of an existing cancer. Although most IARC laboratory-based research has been driven by the upstream perspective, with the ultimate goal of preventing cancer by avoiding its causes, the downstream perspective has also been pursued.

Much of the molecular pathology research developed by Paul Kleihues and Hiroko Ohgaki centres on improving the definition and classification of brain tumours with respect to the biology and – most relevantly – the clinical outlook of patients. As an example, the figure shows how complex patterns of molecular markers can differentiate glioblastomas – the most common and most aggressive brain tumours in humans – into subtypes with markedly different clinical durations, ranging from a few months to several years.



This schematic diagram portrays how glial progenitors – the non-nerve cells that support and protect nerve cells in the brain – give rise to different subtypes of glioblastoma through various sequences of genetic alterations. Primary glioblastomas usually have a short clinical history of 3–6 months. Secondary glioblastomas tend to have a clinical duration of a few years.

Another promising avenue of investigation is the study of microRNAs, small RNA molecules that are involved in the regulation of protein synthesis. A recent IARC study showed that lung cancer cells secrete microRNAs into the blood, making these molecules potential tools for very early diagnosis of the tumour.

For the investigation of predictors of disease, the biological specimens of the EPIC study are once again an eminently suitable resource. A recently published EPIC-based study found a significantly lower level of pre-diagnostic immunoglobulin E levels in subjects who subsequently developed chronic lymphocytic leukaemia.

These more recent studies with a new generation of biomarkers reinforce the more general point that the continued approach of interdisciplinary research has much to contribute to understanding the causes of cancer and how to prevent it.