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Chemical carcinogenesis

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ABSTRACT

The use of chemical compounds benefits society in a number of ways. Pesticides, for instance, enable foodstuffs to be produced in sufficient quantities to satisfy the needs of millions of people, a condition that has led to an increase in levels of life expectancy. Yet, at times, these benefits are offset by certain disadvantages, notably the toxic side effects of the chemical compounds used. Exposure to these compounds can have varying effects, ranging from instant death to a gradual process of chemical carcinogenesis. There are three stages involved in chemical carcinogenesis. These are defined as initiation, promotion and progression. Each of these stages is characterised by morphological and biochemical modifications and result from genetic and/or epigenetic alterations. These genetic modifications include: mutations in genes that control cell proliferation, cell death and DNA repair – i.e. mutations in proto-oncogenes and tumour suppressing genes. The epigenetic factors, also considered as being non-genetic in character, can also contribute to carcinogenesis via epigenetic mechanisms which silence gene expression. The control of responses to carcinogenesis through the application of several chemical, biochemical and biological techniques facilitates the identification of those basic mechanisms involved in neoplasic development. Experimental assays with laboratory animals, epidemiological studies and quick tests enable the identification of carcinogenic compounds, the dissection of many aspects of carcinogenesis, and the establishment of effective strategies to prevent the cancer which results from exposure to chemicals.

Key words: cancer stages,carcinogenesis evaluation, chemical carcinogens, chemical carcinogenesis.

INTRODUCTION

Public opinion considers cancer to be an increasingly threatening disease, affecting people of all ages. After cardiovascular diseases, it is the second cause of death amongst the global population (Huff 1994, Weisburger 1999). People tend to accept cancer with stoicism and submit themselves to prolonged periods of treatments, which are not always effective (Weisburger 1999). The word carcinogenic was defined as the capacity of a compound to unchain the process of cancer development in man and animals under the appropriate conditions, by acting on one of several organs or tissues (Gomes-

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Carneiro et al. 1997, Huff 1999). With the discovery of different mechanisms involved in carcinogenesis, this definition is now incomplete (Butterworth and Bogdanffy 1999). From an experimental point of view, a compound is considered carcinogenic when its administration to laboratory animals induces a statistically significant rise in the incidence of one or more histological types of neoplasia, compared with the animals in the control group which are not exposed to the substance (Gutiérrez and Salsamendi 2001).

The factors responsible for cancer development are classified as exogenous and endogenous (Camargo et al. 1999, Gutiérrez and Salsamendi 2001). The first group includes nutritional habits (food preservation and preparation), socio-economic status, lifestyle, physical agents (ionising and non-ionising radiation), chemical compounds (natural and synthetic) and biological agents (*Helicobacter pylori*, Epstein Barr virus, human T lymphotropic viruses I and II, human papilloma virus and the hepatitis B virus, parasites such as *Schistosoma haemotobium*, *Clonorchis sinensis* and *Opisthorchis vivarium*; growth factors) (Pitot and Dragan 1991, Barrett and Anderson 1993, Farmer 1994, Weisburger 1999, Minamoto et al. 2000, Lutz 2002). Unhealthy lifestyle habits such as: excess alcohol consumption; inhalation of tobacco and related products; the ingestion of certain foods and their contamination by mycotoxins; are responsible for higher incidences of certain types of neoplasias in a number of population groups (Gomes-Carneiro et al. 1997, Weisburger 1999, Gutiérrez and Salsamendi 2001). Endogenous factors include immune system damage and inflammation caused by uncertain aetiology (e.g. ulcerative colitis, pancreatitis, etc.), genetic makeup, age, endocrine balance and physiological condition (Cohen et al. 1991, Barrett and Anderson 1993, Huff 1994, Koivusalo et al. 1994, Weisburger 1999, Minamoto et al. 2000, Gutiérrez and Salsamendi 2001, Dewhirst et al. 2003, Ohshima et al. 2003, 2005).

Epidemiological studies of cancer incidence demonstrated that the risk of developing cancer varies between population groups and these differences are associated with lifestyle factors and habits (Garner 1998, Lai and Shields 1999, Gutiérrez and Salsamendi 2001). Population migration has resulted in the development of types of cancer typical of particular geographical areas (King et al. 1995, Gutiérrez and Salsamendi 2001).

The relationship between chemical substances in the workplace and the development of certain neoplasias in various occupational groups led to the conception of experimental models to better understand the biopathological processes inherent to carcinogenesis (Weinstein 1991, Cohen et al. 1992, Gutiérrez and Salsamendi 2001).

Boveri laid down the genetic basis of neoplasic development for the first time in 1914 with his theory of somatic mutation in cancer cells. However at the time, experts in the area of chemical carcinogenesis attributed little importance to this hypothesis, considering it to be pure speculation, instead choosing to put their faith in the lesser knowledge already available (Weisburger 1999). Between 1980 and 1990, the discoveries made via the molecular biology of proto-oncogenes and tumour suppressor genes strengthened the case behind this supposition (Cohen 1998). Neoplasic development bases itself on the existence of several genetic mutations, despite the number not being known. In most of the cases it is assumed to vary between tissues and between different species (Grisham et al. 1984, Cohen 1995, 1998, Simons 1995, van Leeuwen and Zonneveld 2001, Lutz 2001, Gutiérrez and Salsamendi 2001). During cell division, spontaneous genetic errors occur. It is estimated to happen at a frequency of around 10^{-5} to 10^{-6} through nucleotides and cell division. If the damage reaches a gene responsible for neoplasic development then the probability of developing cancer will be greater (Cohen 1995).

A cancer is made up of billions of cells, all originating from an initial cell which multiplies clonally, escapes to apoptosis and accumulates genetic (and/or epigenetic) alterations which converge into a neoplasic cell (Trosko 2001). The blocking of apoptosis in the face of significant genetic damage can ease the accumulation of aberrant cells and it can become a critical point in malignance pathogenesis (Nguyen-ba and Vasseur 1999, Qu et al. 2002).

Neoplasias can be classified as benign or malign depending on their cellular characteristics. The constituent cells of a malign neoplasia show yet more changes in cell biology (Fig. 1). They proliferate autonomously, differentiate themselves, invade adjacent tissues and frequently metastasize on tissues that are not related to the

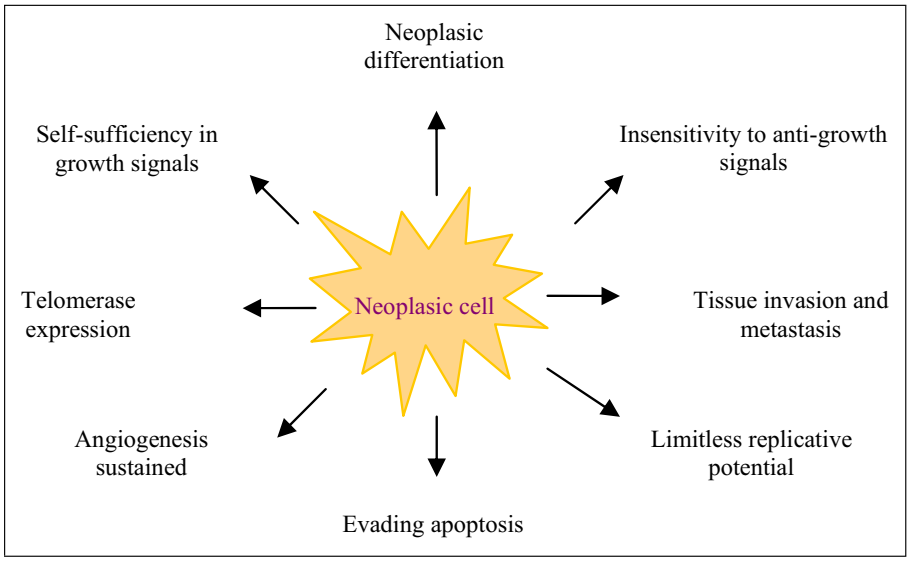

Fig. 1 – Malignant cell characteristics.

primary neoplasia (Hanahan and Weinberg 2000, Shacter and Weitzman 2002). Cells, which are part of benign neoplasias, grow more slowly, and in general, they do not disturb normal tissue function, unless they compress vital structures (Player et al. 2004). The histopathological observation of neoplasias, be they induced or spontaneous, enables us to better evaluate carcinogenesis, but it may not be enough to identify more subtle alterations such as molecular changes (Huff 1992, Maronpot 1996).

This review aims to describe of different events involved in chemical carcinogenesis. So, our work starts with a historical perspective of the study of chemical carcinogenesis; we will describe the different stages involved in carcinogenesis; the absorption and metabolism of chemical carcinogens. We will classify different types of carcinogens in function of their active mechanisms and we will describe the molecular targets of carcinogens. Finally, we will describe a selection of the methods available for evaluating the carcinogenic potential of chemical compounds.

HISTORICAL PERSPECTIVE OF CHEMICAL CARCINOGENESIS STUDY

Cancer was described for the first time by Hippocrates as 'karkinos'. Galeno introduced the word neoplasia only in the II century; he defined it as the growth of a body area adverse to nature (Gutiérrez and Salsamendi 2001). Edwin Smith's papyruses, dating from the XVII century, describe breast tumefaction.

According to Hayes (1995), it was the English surgeon Percivall Pott who first recognized in 1775 the casual relationship between exposure to environmental substances and neoplasic development. This author described the occurrence of cancerous alterations in the skin of the scrotum of London chimney sweeps as a consequence of repeated localised contamination with soot. Some years later, and based on these observations, a guide distributed to Danish chimney sweeps recommended that these professionals take a daily bath to avoid such an occurrence (Hayes 1995, Gutiérrez and Salsamendi 2001). Still in the XVIII century John Hill observed a high proportion of nasal mucosa cancer in his patients, and traced it to the localised long-term exposure to snuff. In 1890, a high incidence of bladder cancer in chemical and rubber industry workers was observed across Europe. (Cohen and Ellwein 1991, Gomes-Carneiro et al. 1997, Garner 1998, Dybdahl et al. 1999, Huff 1999, Bertram 2001). By the end of the nineteenth century it had become evident that occupational exposure to certain chemicals or mixtures of chemicals had carcinogenic effects (Luch 2005). The all-important next step was to systematically investigate and reproduce these diseases in experimental surroundings. The first experimental work on chemical carcinogenesis was

carried out in 1915 by the pathologist Katsusaburo Yamagiwa and his assistant Koichi Ichikawa (Yamagiwa and Ichikawa 1918). They rubbed rabbit ears with coal tar and observed the development of papillomas and carcinomas. Meanwhile, others researchers studied carcinogenesis of the bladder, liver, kidney, pancreas and lung using laboratory animals. Its success laid the foundations of the experimental use of animals in the study of human diseases (Toth 2001). Later, Beremblum and Shubik used polycyclic aromatic hydrocarbons and croton oil to study skin carcinogenesis in mice and demonstrate that cancer development includes several stages (Beremblum and Shubik 1947). When applied in low doses, none of these substances have carcinogenic properties by themselves. Yet, when mixed and in equal doses, they induced neoplasic development. The order of exposition to these substances was fundamental for carcinogenesis. Neoplasias developed only when the hydrocarbons were used first and then the croton oil, never the other way around. These authors felt that the carcinogenic action of these substances was responsible for converting normal cells into neoplasic cells. For them, carcinogenesis was a complex process including one phase called initiation and another called promotion, with one or more genetic changes necessary for cancer development. During the next decade, Foulds (1954) introduced the term progression by studying breast adenocarcinoma in female mice. In the pre-Watson and Crick era, before carcinogens were known to bind to DNA, the cancers produced by chemical carcinogens were believed to be due to their interaction with proteins in specific tissues (Miller and Miller 1952). By the end of the 1960s, increasing evidence pointed to a correlation between the DNA binding capacity of a particular carcinogen and its biological potency (Luch 2005).

STAGES OF CARCINOGENESIS

Studies conducted using animal models, "in vitro" studies and epidemiologic assays enabled investigators to conclude that neoplasic pathogenesis is a complex process which can be divided into three distinct stages, from an operational point of view. These are: initiation, promotion and progression (Foulds 1954, Grisham et al. 1984, Cohen 1991, Mehta 1995, Hasegawa et al. 1998, Gutiérrez and Salsamendi 2001, Trosko 2001).

Changes in the genome's structure occur across the three stages of neoplasic development (Simons 1995, Pitot 2001, Luch 2005). Changes in gene expression also take place during the promotion stage, with selective proliferation of initiated cells and the development of pre-neoplastic cells (Grisham et al. 1984, Gutiérrez and Salsamendi 2001). During initiation and promotion, apoptosis and cell proliferation can occur at different rates, while remaining balanced. During progression, this balance is modified and from there malignancy arises (Mehta 1995) (Fig. 2).

Human life is led under very different conditions from these experimental procedures. Although the process of carcinogenesis is similar for man and experimental animals, the different chemical compounds to which humans are exposed throughout their lives alter the speed of the process and the frequency of mutation, the speed of cell growth and the phenotypical expression of the changed genes. On the other hand, the individual's susceptibility and their defence mechanisms have their own interaction, which modifies each of the neoplasic stages.

Initiation

The first stage of carcinogenesis has been labelled initiation since 1947 (Beremblum and Shubik 1947). The conclusions reached from several experiments enabled the conclusion to be drawn that initiation is caused by irreversible genetic changes which predispose susceptible normal cells to malign evolution and immortality (Beremblum and Shubik 1947, Stenbäck et al. 1981, Butterworth et al. 1992, Mehta 1995, Dybing and Sanner 1999, Trosko 2001, 2003, Shacter and Weitzman 2002). The initiated cell is not a neoplasic cell but has taken its first step towards this state, after successive genotypical and phenotypical changes have occurred (Trosko 2003). From a phenotypical perspective, the initiated cell is similar to the remaining cells. It undergoes mutations and these induce proliferation but not differentiation (Trosko 2001).

DNA damage has been well established as the event which kick-starts chemical carcinogenesis (Santella et al. 2005). DNA damage can be repaired by enzymatic mechanisms (Bertram 2001, Jeng et al. 2001, Shacter and Weitzman 2002). Cells which are proliferating have less time to repair the damaged DNA and remove co-

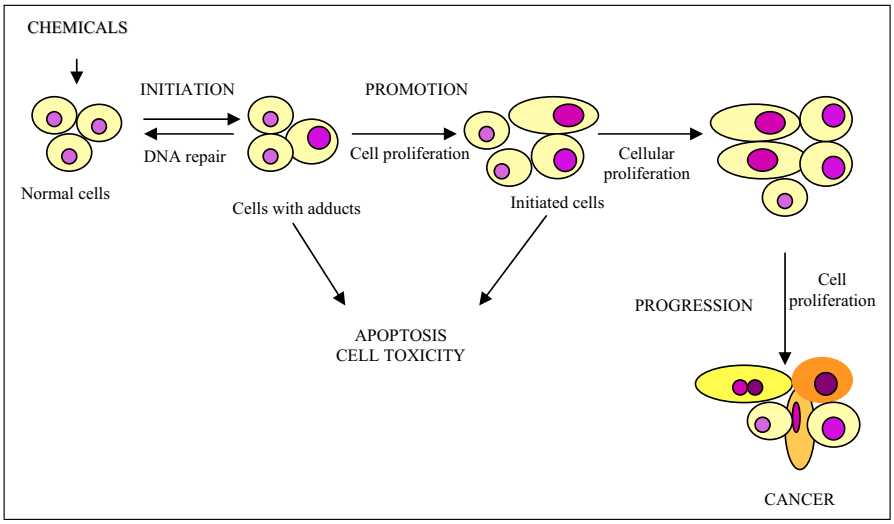

Fig. 2 – Chemical carcinogenesis stages and the occurrences involved in each one.

valent bonds that chemicals establish with the DNA – known as adducts (Heidelberger 1977, Richardson et al. 1986, Frowein 2000).

At this stage, the initiated cells can remain latent for weeks, months or years, or they can grow in an autonomous and clonal fashion (Scott et al. 1984, Dybing and Sanner 1999, Player et al. 2004). This initiation process ensures that cellular division remains symmetrical by creating two new initiated cells (Trosko 2003). The clonal expansion of initiated cells results from a mitogenic process caused by an increase in the number of new cells and apoptosis inhibition, which prevents initiated cells from dying off (Trosko 2001).

The increase in DNA damage is specifically important to stem cells, because they survive for a long time and exist in several tissues (Potter 1978, Simons 1999, Trosko 2001, Williams 2001). In 1978, Potter explained that neoplasic cells could display a phenotype established between the embryonic aspect and the terminal differentiation, and that all neoplasic cells had monoclonal origin from a stem cell. By definition, stem cells are immortal cells until they differentiate, or death is induced. If we delay their differentiation they become initiated and accumulate in tissues as clones of abnormal cells (Trosko 2003). Although stem cells are not identifiable in most tissues, it is believed that every tissue has a population of stem cells (Player et al. 2004).

Initiation is a fast, irreversible phenomenon and is transmitted to daughter cells (Farber 1984). Cell proliferation is essential for this stage, if cellular division occurs before DNA repair systems can act then the injury becomes permanent and irreversible. Initiation is an additive process, neoplasic development depends on the carcinogenic dose, increasing the dose increases the incidence and the multiplicity of resultant neoplasias and reduces the latent period of its manifestation. Not all cells of a living organism exposed to an initiator agent will be initiated even if they have suffered mutations, and the genes that regulate the terminal differentiation must also be mutated (Farber 1984, Yuspa and Poirier 1988, Klaunig et al. 2000, Trosko 2001).

Spontaneously initiated cells exist in all living organisms (Gomes-Carneiro et al. 1997, Trosko 2001). Initiation can begin with spontaneous mutations, supported by normal occurrences such as DNA depurination and deamination. Errors in DNA replication are also associated with initiation. Although spontaneous initiation is less common than induced initiation, its existence has been confirmed by the occurrence of spontaneous neoplasias in laboratory animals (Pitot and Dragan 1991, Gomes-Carneiro et al. 1997).

PROMOTION

The concept of promotion was introduced when chemical substances with low carcinogenic activity were discovered, which were still able to induce the develop-

ment of cancer under experimental conditions (Beremblum and Shubik 1947).

Promoter compounds do not interact directly with DNA and unchain biological effects without being metabolically activated (Yuspa et al. 1983, Butterworth et al. 1992, Weisburger 1998, Williams 2001). These agents increase cell proliferation in susceptible tissues, contribute towards fixing mutations, enhance alterations in genetic expression and cause changes in cellular growth control (Mehta 1995, Gomes-Carneiro et al. 1997). On the other hand, these promoters may indirectly damage DNA by oxidation (Gutiérrez and Salsamendi 2001). At first, these occurrences were associated with epigenetic mechanisms, but nowadays it is widely agreed that promotion also involves genetic changes (Simons 1995, Hanahan and Weinberg 2000).

Promoters delay the natural inhibition of the quiescent cells or in G_0 by gap junctions (Barrett and Anderson 1993, Simons 1999, Bertram 2001, Trosko 2001). The promoters' most important activity is mitogenesis – genotoxical and mutational actions are not necessary at this stage (Pitot and Dragan 1991). The promoter must be present for weeks, months and years in order to be effective and its effectiveness depends on its concentration in the target tissue (Butterworth et al. 1992). Promotion is a reversible stage, after a promoter's disappearance a regression in cell proliferation can occur, probably by apoptosis. It is a stage that can be moulded up by physiological factors and therefore limit the extent of experimental carcinogenesis. Some promoter agents are specific for a particular tissue, but others act simultaneously upon several tissues (Yuspa et al. 1983, Scott et al. 1984, Yuspa and Poirier 1988, Gutiérrez and Salsamendi 2001).

In studies of chemical carcinogenesis with prolonged exposure and using high doses almost all of the promoter agents induce neoplasias without initiation (Pitot and Dragan 1991, Gutiérrez and Salsamendi 2001). Exposure to phenobarbital, benzene, asbestos, and arsenic even without the previous application of initiator agents leads to neoplasic development (Melnick et al. 1996, Trosko 2001). This contradiction has two possible explanations: either the genotoxic effect was not identified by mutagenicity and genotoxicity assays, or the initiated cells emerged spontaneously. In this last case we may consider that the promoter has an indirect effect – by increasing the frequency of cellular division it encourages the appearance of errors in DNA replication, as well as mutations.

Not all cells exposed to promoters take part in the promotion stage, only cells which are stimulated to divide, that are undifferentiated, and have survived apoptosis, can contribute to instability between growth and cell death and lead to the appearance of a malign neoplasia (Trosko 2001).

Progression

The sequence of lesions identified, via histopathology, between initiation and promotion are designated as preneoplastic lesions and/or benign neoplasias (Gutiérrez and Salsamendi 2001). Their transformation into malign lesions is the last of the stages of carcinogenesis and is the most extended – it is labelled progression (Klaunig et al. 2000, Williams 2001). In progression, a neoplasic phenotype is acquired through genetic and epigenetic mechanisms (Shacter and Weitzman 2002). During progression, cell proliferation is independent from the presence of stimulus (Lutz 2000, Gutiérrez and Salsamendi 2001).

Progression is characterised by irreversibility, genetic instability, faster growth, invasion, metastization, and changes in the biochemical, metabolical and morphological characteristics of cells (Pitot and Dragan 1991, Butterworth et al. 1998, Loeb 1998, Klaunig et al. 2000, Gutiérrez and Salsamendi 2001, Dixon and Kopras 2004).

Angiogenesis, as an epigenetic occurrence, is essential to neoplasic progression. The acquisition of an angiogenic phenotype precedes the development of characteristics that contribute to malignancy and its inhibition delays neoplasic development (Hawighorst et al. 2001).

ABSORPTION AND METABOLISM OF CHEMICAL CARCINOGENS

Following exposure, chemical carcinogens may be absorbed in a number of ways (oral, inhalator, cutaneous, and injection) and distributed across several tissues (Connoly et al. 1988). Absorption depends on the physicochemical properties of the substance and can take place via passive or active transport. The substances absorbed

orally pass through the liver and only then are they distributed in the body; those absorbed in the lung are distributed by the blood before reaching the liver at a later stage (King et al. 1995, van Leeuwen and Zonneveld 2001). Those carcinogenic compounds classified as direct act directly on DNA, but most require enzymatic conversion and are thus labelled as indirect or procarcinogens (Sarasin and Meunier-Rotival 1976, Hayes 1995, Lai and Shields 1999, Klaunig et al. 2000, Oesch et al. 2000, Poirier et al. 2000, Luch 2005). Metabolic activation is controlled by phase I reactions, while phase II reactions protect the body through the transformation of activated compounds into inert products which are easily eliminated from the body (Fig. 3) (Hayes 1995, Bartsch and Hietanen 1996, Mostafa et al. 1999, Klaunig et al. 2000, Gonzalez and Kimura 2001, van Leeuwen and Zonneveld 2001, Park et al. 2005).

The performance of metabolic enzymes is essential for understanding chemical carcinogenesis and learning the differences between species as far as their susceptibility to neoplasic development is concerned (Sarasin and Meunier-Rotival 1976, Lai and Shields 1999, Guenguerish 2000, 2001, Gonzalez 2001). The enzymes in phase I participate in the reactions of oxidation, reduction and hydrolysis, and are classified as oxidoreductases (cytochrome P450 dependent monooxygenases, flavine monooxygenases, cyclooxygenases and alcohol dehydrogenase) and hydrolases (epoxide hydrolases) (Hayes 1995, Garner 1998, Galati et al. 2000, Oesch et al. 2000, Garcea et al. 2003). Phase II enzymes participate in the conjugation and inactivation of chemical carcinogens and include transferases (glutathione S-transferases, N-acetyltransferases, UDP-glucuronosyltransferases, sulphotransferases) (Oesch et al. 2000, Guengerich 2000, Gonzalez 2001). Although these enzymes were originally only thought to be involved in the detoxification stages of biotransformation, they can also contribute to the activation of certain procarcinogens *in vivo* (Luch 2005).

Metabolic activation occurs predominantly in the liver at the plain endoplasmic reticulum where the cytochrome P450 is more abundant, and to a lesser degree in the bladder, skin, gastrointestinal system, oesophagus, kidneys, and lungs (Bartsch and Hietanen 1996, Mostafa et al. 1999, Guengerich 2001, van Leeuwen and Zonneveld 2001, Oda 2004). During this phase the cytochrome P450 mono-oxygenases introduces a reactive polar group into the carcinogenic, making it lipophylic. It then converts it into a powerful electrophilic product capable of establishing adducts with DNA (Straub and Burlingame 1981, Lai and Shields 1999, Galati et al. 2000, Park et al. 2005). Phase II reactions are catalysed by hepatic and extra hepatic, cytoplasmic and cytochromic enzymes, acting separately or joined together (Gonzalez 2001). Conjugation reactions enable these enzymes to decompose the polar group in glucose, amino acids, glutathione and sulphate, which are less toxic metabolites that are more soluble in water and more easily expelled by the urine and bile (Galati et al. 2000, Oesch et al. 2000, Gonzalez and Kimura 2001, van Leeuwen and Zonneveld 2001).

Peroxidations also occur parallel to metabolic reactions with the continuous production of reactive oxygen species (ROS) (Weisburger 1999, Klaunig et al. 2000, Ohshima et al. 2005). These radicals are associated with several chronic diseases including chemical carcinogenesis (Klaunig et al. 2000). The ROS damage DNA, RNA, and proteins by chemical reactions such as oxidation, nitration/nitrosation and halogenation. This leads to an increase in mutations and alterations in the functions of important enzymes and proteins (Park et al. 2005). Several experiments have proved that chemical compounds, which create ROS in excess, encourage initiation, promotion and neoplasic progression through genotoxicity (Galati et al 2000, Shacter and Weitzman 2002). The impact of the ROS controlled by a cellular mechanism that operates at different levels: metabolism; reactions that maintain the redox balance in cells; transduction of the signal regulator of oxidation and DNA reparation (Bolt et al. 2004).

Park et al. (2005) says that the same enzyme may have the capacity to activate one chemical and deactivate another, all depending on its chemical structure. The specificity of the activation systems of different tissues regulate neoplasic development and is dependent on genetic polymorphism, which requires the expression and distribution of the enzymes involved in phase I and II reactions, and the resulting susceptibility to cancer development (Schut and Castonguay 1984, Hayes 1995, Henglster et al. 1998, Mostafa et al. 1999, Dybing

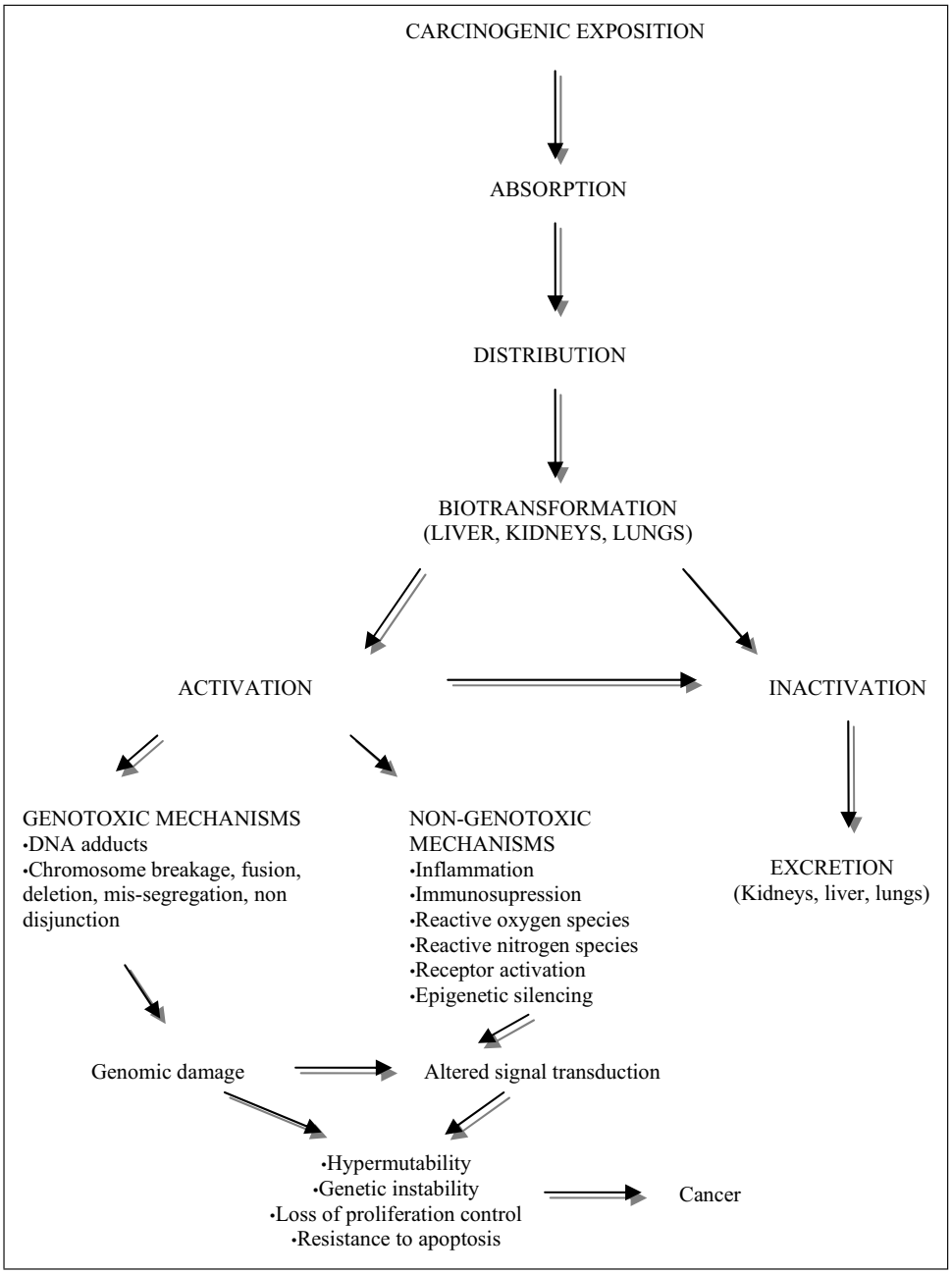

Fig. 3 – Metabolic activation of chemical compounds and genotoxic and non-genotoxic effects of carcinogens.

and Sanner 1999, Gonzalez 2001, Gonzalez and Kimura 2001, Gutiérrez and Salsamendi 2001, Lutz 2002). People with a high quantity of phase I and a low quantity of phase II enzymes have a higher probability of synthesising intermediate compounds and exhibiting more DNA damage (Rojas et al. 2000).

The previously described metabolic methods are equally important for both humans and animals, although there exist qualitative and quantitative differences between them. These have lead to incorrect interpretations when animal models are used in the research and analysis of carcinogenic properties of chemical compounds (Guengerich 2000, Gonzalez 2001, Gonzalez and Kimura 2001).

Several studies have been developed in order to evaluate the differences between several exogenous and endogenous factors on individual susceptibility to carcinogenesis (Table I) (Barrett 1993, Bartsch and Hietanen 1996, Maronpot 1996, Lutz 1998, 1999, Ishikawa et al. 2001, Miller et al. 2001).

TABLE I
Factors that control and change chemical carcinogenesis.

Age	Virus
Sex	Diet, nutrition and life style
Animal species	Genetic constitution
Endocrine system	Anticancer drugs
Immune system	Metabolic ways
Trauma	DNA repair
Radiation	

CARCINOGENIC CLASSIFICATION

Carcinogenic classification is by no means consensual (Butterworth and Bogdanffy 1999, Bolt et al. 2004). It is not easy to incorporate a carcinogenic compound into a certain group because the information obtained from different studies is increasingly complex (Pitot and Dragan 1991, Butterworth et al. 1992). Some authors classify them in function of their participation in each of the stages of carcinogenesis. In this way, incomplete carcinogens are mutagenic chemicals that instigate irreversible DNA damage (Mirsalis et al. 1990, Pitot and Dragan 1991). A complete carcinogen displays properties of both initiators and promoters simultaneously depending on the dosage and exposure time (Pitot and Dragan 1991, Farmer 1994, Hasegawa et al. 1998, Trosko 2001).

Other authors classify chemical carcinogens in function of their mechanisms of action as being genotoxic and non-genotoxic (mitogenic and cytogenic) (Cohen and Ellwein 1991, Butterworth et al. 1992, Nguyen-ba and Vasseur 1999, Klaunig et al. 2000, Williams 2001). The knowledge about the mechanism of action of non-genotoxic carcinogens is known to be inferior to that of genotoxic carcinogens.

Genotoxic carcinogens are complete carcinogens and qualitatively and quantitatively change a cell's genetic information (Trosko 2001). They exhibit a direct analogy between their structure and activity, are mutagenic on in vitro assays, are active in high doses, and may affect several animal species, and damage different organs (Klaunig et al. 2000, Gutiérrez and Salsamendi 2001, Luch 2005). In high doses, they cause toxicity and cell proliferation, increasing DNA replication and influencing its carcinogenic activity (Cohen 1998). Following transmembranar diffusion they are metabolized in electrophilic compounds that enter the nucleus and interact with nucleophilic sites (DNA, RNA and proteins) changing their structural integrity and establishing covalent bonds known as adducts (Miller and Miller 1975, Straub and Burlingame 1981, Cohen et al. 1992, Ashby 1996, Weisburger 1998, Frowein 2000, Bertram 2001, Lutz 2001, Williams 2001, Baird and Mahadevan 2004). The formation of adducts constitutes the first critical step of carcinogenesis and if these are not repaired before DNA replication then mutations may occur in the proto-oncogenes and tumour suppressor genes, which are essential for the initiation stage (Sobels 1975, Barrett and Wiseman 1987, Farmer 1994, Lutz 2001, Williams 2001, Li et al. 2005). The number of adducts formed by carcinogens is changeable and each of them may cause a specific damage to DNA (Straub and Burlingame 1981, Farmer 1994, Otteneder and Lutz 1999). Mutations linked to adducts can appear through deletion, frameshift, or by nucleotide substitution (Garner 1998). Mutations cause an undefined number of cell changes, translated into aberrant protein expression and in changes in cell cycle control. Adducts assume importance in chemical carcinogenesis because of the way they change DNA, possibly inducing an incorrect transcription and causing mutations of the new DNA chain. The existence of many adducts can break the DNA chain, causing mutation or loss of genetic material (Cohen 1995, Hayes and Pulford 1995, Trosko 2001). Adduct repair is coordinated by several enzymes and controlled by different genes. It can be done via the excision of bases, or nucleotides, recombined repair or mismatch repair (Farmer 1994, Moustacchi 1998, Miller et al. 2001, Hanawalt et al. 2003).

The identification of adducts suggests that chemical carcinogens are absorbed, metabolized and distributed by tissues, thus fleeing from the body's detoxification and repair mechanisms (Garner 1998, Airoldi et al. 1999,

Guengerich 2000). The identification and analysis of adducts can be carried out using marked radioactive carcinogens, those most-commonly used are ^{14}C and tritium, each adduct can be identified by their 10^6 or 10^7 nucleotides (Garner 1998). However, the most used techniques are immunoassays with ^{32}P, gaseous chromatography associated with mass spectrometry and HPLC associated with fluorescent spectroscopy (Farmer 1994, Airoldi et al. 1999). There are also monoclonal and polyclonal antibodies available on the market which are used to identify adducts by immunohistochemistry (Santella et al. 2005). There is a positive correlation between the quantity of adducts detected in animal models and the number of neoplasias developed (Yuspa and Poirier 1988, Williams 2001, Baird and Mahadevan 2004).

Non-genotoxic carcinogens act as promoters and do not need metabolical activation. They do not react directly with DNA, do not raise adducts and show negative on mutagenicity tests carried out in vivo and in vitro (Butterworth et al. 1992, Melnick et al. 1996, Butterworth and Bogdanffy 1999, Klaunig et al. 2000, Gonzalez 2001, Williams 2001). These compounds modulate growth and cell death, potentate the effects of genotoxic compounds, do not show a direct correlation between structure and activity, and their action is limited by their concentration. They are tissue- and species-specific (Farmer 1994, Melnick et al. 1996, Gomes-Carneiro et al. 1997, Butterworth and Bogdanffy 1999, Klaunig et al. 2000). Melnick et al. (1996) states that exposure to these compounds favours the synthesis of other substances responsible for neoplasic development. These compounds promote effects on target cells which indirectly unchain neoplasic transformation or increase neoplasic development from genetically changed cells (Williams 2001). Non-genotoxic carcinogens are classified as cytotoxic and mitogenic in function of whether their activity is mediated by a receptor or not (Cohen 1991, Cohen et al. 1992, Butterworth and Bogdanffy 1999). Mitogenic compounds such as phorbol esters, dioxins, and phenobarbital induce cell proliferation in target tissue through interaction with a specific cellular receptor (Cohen et al. 1992). Cytotoxic carcinogens cause cell death in susceptible tissues followed by compensatory hyperplasia, taking chloroform as an example (Cohen et al. 1991, Butterworth et al. 1992, Klaunig et al. 2000). If the carcinogen dose is high, some cells cannot survive. The more that nearby cells increase the number of cell divisions through regenerative procedures, the more likely it is that they will end up being prematurely recruited for the cell cycle and that the time available for reparation DNA will be inferior – this increases the probability of mutations occurring (Cohen 1991, Melnick et al. 1996). On the other hand, necrosed cells are destroyed by the immune system and ROS, reactive nitrogen species (RNS), and proteolytic enzymes are produced (Lutz 1998, Ohshima et al. 2005). When production of these ROS and RNS exceeds the cellular anti-oxidant capacity, it may cause oxidative damages to lipids, proteins, carbohydrates, and nucleic acids, leading to carcinogenesis and cell death (Ohshima et al. 2005). Mitogenic compounds need to be present in certain concentrations to promote their activity. Contrastingly, the action of non-cytotoxic compounds is independent of their concentrations (Butterworth et al. 1992, Butterworth and Bogdanffy 1999).

Chemical carcinogens can be classified into several groups, on Table II we brought them together under the following headings: Group, compound, mechanism of action, and affected organs/cancer type.

As we mentioned before, the classification of the carcinogenic compounds according to their mechanism of action continues to cause controversy. Bolt et al. (2004) propose the division of genotoxic compounds into two groups: those which react with DNA, and genotoxic at a chromosomal level. Compounds, which react with DNA, are subdivided into three different groups: initiators (with unlimited doses), borderline, and weak genotoxic (they act by secondary mechanisms) (Fig. 4).

Chemical carcinogens can have additional synergic or antagonistic effects when simultaneously presented in different metabolic ways (Schmahl 1976, Lutz 2001). The synergy between smoking and exposure to asbestos favours lung cancer development as a consequence of chronic inflammation and compensatory cell proliferation. This antagonism may be exemplified by the protective action of fruit and vegetables in the modulation of individual susceptibility to neoplasic development (Lutz 2001, 2002).

TABLE II
Chemical carcinogens.

Group	Compound	Mechanism of action	Affected organs/ Cancer type
Polycyclic aromatic hydrocarbons	Benzo[a]pyrene Polychlorinated biphenyls (Luch 2005)	Form adducts with purine bases of DNA, mainly resulting on transversions	Skin, lungs, stomach Liver skin
Aromatic amines/amides	2-Acetylaminofluorene 4-Aminobiphenyl 2-Naphthylamine (Luch 2005)	Genotoxic compounds, increase the rate of cell duplication	Liver, bladder Bladder Bladder
Aminoazo dyes	o-Aminoazotoluene *N*, *N*-dimethyl-4-aminoazobenzene (Golka et al. 2004)	Forms adducts with DNA and with haemoglobin	Liver, lungs, bladder Lungs, liver
N-nitroso compounds	N-Nitrosodimethylamine (Drablos et al. 1998)	Form adducts at N- and O-atoms in DNA bases	Liver, lungs, kidneys
Carbamates	N-methylcarbamate esters (Wang et al. 1998)	Chromosome aberration, gene mutation, cell transformation	Experimental results showed liver, kidneys and tests degeneration
Halogenated compounds	Trichloroethylene (Lock et al. 2007)	Somatic mutations, modification of cell cycle pathways	Experimental results showed kidney, liver and lung cancer
Natural carcinogens	Aflatoxin B1 (Wild et al. 1986) Asbestos (Luch 2005)	Forms adducts with guanine, react with RNA and proteins	Liver Lungs
Metals	Arsenic (Shi et al. 2004) Cadmium (Hartwig et al. 2002) Nickel (Costa et al. 2003)	Oxidative stress Inhibit DNA repair pathways and nucleotide-excision repair Histone acetylation and DNA hypermethylation	Skin, lungs, liver Lungs, prostate, kidneys Lungs, nasal cavity
Anticancer drugs	Alkylating agents (Luch 2005)	Interstrand and/or intrastrand cross-links	Leukaemia

EPIGENETIC MECHANISMS INVOLVED IN CHEMICAL CARCINOGENESIS

The most well understood epigenetic mechanisms involve DNA methylation and histone acetylation, methylation, and phosphorylation (Fig. 5). Demethylation of promoter regions at the CpG sequences can lead to an over-expression of proto-oncogenes, and silencing of gene expression can occur as a result of hypermethylation, sometimes leading to chromosome condensation (Klaunig et al. 2000). There appears to be a relationship between DNA methylation and histone modifications; patterns of histone deacetylation and histone methylation are associated with DNA methylation and gene silencing. Interestingly, these epigenetic changes in chromatin can also alter the sensitivity of DNA sequences to mutation, thus rendering genes more susceptible to toxic insult (Dixon and Kopras 2004).

MOLECULAR TARGETS OF CHEMICAL CARCINOGENS

The discovery of the ability of oncogenes to induce neoplasic transformation when transfected into immortalized mouse cell lines, initially seemed to answer many

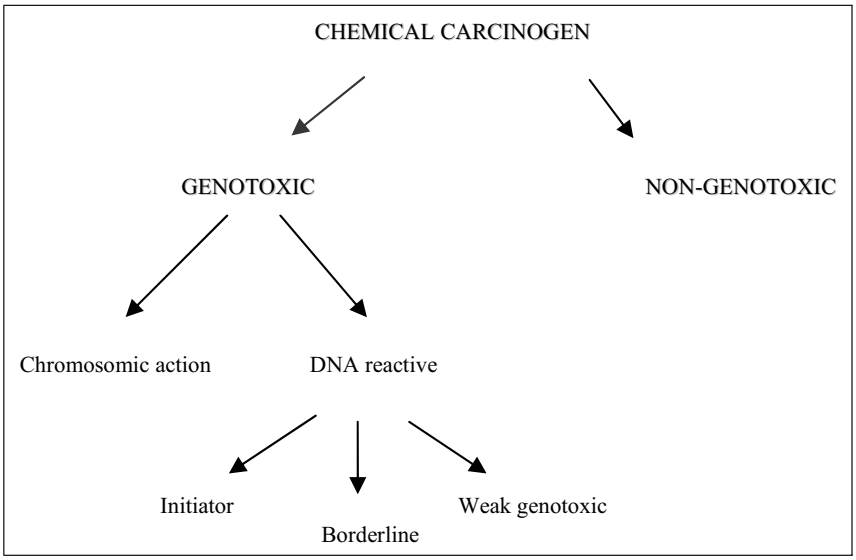

Fig. 4 – New proposal to classify chemical carcinogens.

basic molecular questions about the molecular origins of cancer. However, it soon became clear that this was not the whole picture and that there existed other genes that could influence neoplasic transformation (Bertram 2001). There are several genes which intervene in carcinogenesis – their identification revolutionised chemical carcinogenesis and oncology (Kinzler and Vogelstein 1997, Bertram 2001). Out of all of these, proto-oncogenes, tumour suppressor genes and cell cycle regulator genes assume a particular importance (Mehta 1995, Nguyen-ba and Vasseur 1999, Klaunig et al. 2000). Unlike diseases such as cystic fibrosis or muscular dystrophy, wherein mutations in one gene can cause disease, no single gene defect "causes" cancer. Mammalian cells have multiple safeguards to protect them against potentially lethal effects of cancer gene mutations, and only when several genes are defective does an invasive cancer develop. Thus it is best to think of mutated cancer genes as contributing to, rather than causing, cancer (Vogelstein and Kinzler 2004). Neoplasic development requires errors in cellular defence mechanisms, which are controlled by checkpoints that may forbid the entry of cells with DNA damage into the cell cycle before DNA reparation occurs (blocked at G_1) and the cell divides (blocked at G_2) (Fig. 6) (Khan et al. 1999, Khan and Dipple 2000). The capacity of cells to evade the cellular defence mechanism has an undoubted contribution towards the carcinogenesis (Khan and Dipple 2000).

The tumour suppressor proteins p53; p21 and pRb play crucial roles in cellular protection, because they encourage the blocking of cells at G_1 (Khan et al. 1999). The loss of pRb protein function provokes an increase in the cell proliferation rate and an absence of terminal differentiation. p53 can interrupt the cell cycle at G_1 and go on to repair DNA damage (Melnick et al. 1993, Loeb 1998, Khan and Dipple 2000, Pritchard et al. 2003, Dixon and Kopras 2004). The most prominent and best-studied tumour suppressor is p53, if DNA is damaged then p53 can induce apoptosis in order to maintain the stability of the cells' genome (Klaunig et al. 2000, Hanawalt et al. 2003, Babenko et al. 2006). The loss of p53 during carcinogenesis can predispose preneoplastic cells to accumulate additional mutations by blocking the normal apoptotic response to genetic damages (Klaunig et al. 2000). The loss of p53 function activates proto-oncogenes and inactivates tumour suppressor genes therefore performing an exceptional role in chemical carcinogenesis (Luch 2005). The biological activity of p53 protein is dependent on its ability to bind transcriptional regulatory elements in DNA. The search for critical genes regulated by p53 led to the discovery of the p21 gene. p21 acts as an inhibitor of cyclin-dependent kinases providing a functional link between p53 and cell cycle (Bertram 2001).

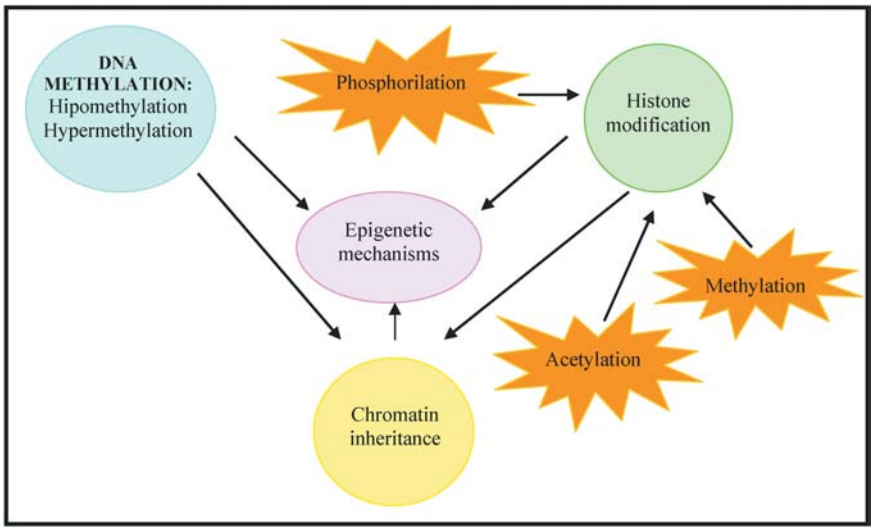

Fig. 5 – Epigenetic mechanisms involved in chemical carcinogenesis.

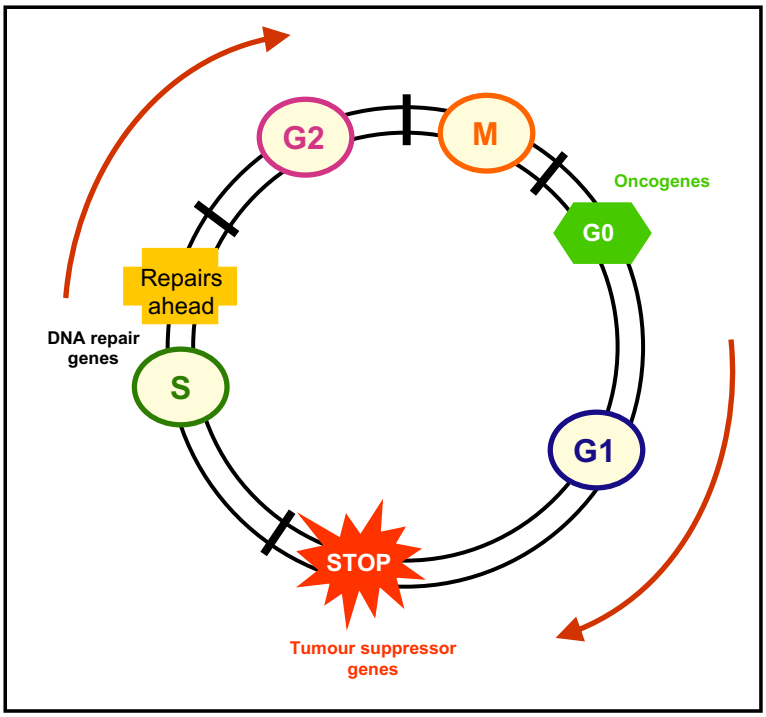

Fig. 6 – Cell cycle and its control by molecular targets (oncogenes and tumour suppressor genes). The cell cycle is a critical process that a cell undergoes in order to copy itself exactly. Most cancers cause mutations in the signals that regulate a cell's cycle of growth and division, namely in oncogenes (which act as dominant mutations) and tumour suppressor genes (that function recessively). Normal cell division is required for the generation of new cells during development and for the replacement of old cells as they die. In normal cells, tumour suppressor genes act as braking signals during G1 to stop or slow the cell cycle before it reaches the S phase. DNA repair genes are active throughout the cell cycle, particularly during G2 after DNA replication and before the chromosomes prepare for mitosis.

A common feature of all the known genetic cancer syndromes is that they are predisposed only to selective types of malignancy. However, many of the genes mutated in these syndromes are ubiquitously expressed, and influence seemingly universal processes such as DNA repair or cell cycle control (Chao and Lipkin 2006). DNA repair is a process which enables a cell to maintain its genome fidelity. There are several routes towards DNA repair. For example, there is excision repair, which consists of both nucleotide excision repair (NER) and base excision repair (BER), mismatch repair (MMR), and double strand break (DSB) repair, as reviewed by Friedberg (2003). Each pathway utilizes unique enzymatic mechanism. In this review we outline the DNA repair processes mediated by p53 family target genes (Fig. 7) once the p53 has been mutated in a very large fraction of tumours from nearly every possible source. In their role as genomic protectors, it is not surprising that the p53 family have a part to play in DNA repair (Fig. 7). The p53 family participate in NER by inducing the expression of GADD45, xeroderma pigmentosum group E gene [XPE] and XPC (Hwang et al. 1999, Tan and Chu 2002, Adimoolam and Ford 2002). GADD45 has also been shown to interact with the core histones and facilitate topoisomerase relaxing of chromatin (Carrier et al. 1999). Defective NER is associated with xeroderma pigmentosum (XP), an autosomal recessive disorder characterized by excessive skin cancers caused by an extreme sensitivity to UV light (Harms et al. 2004).

The mismatch repair pathway is also influenced by the p53 family. p53 and p73 induce the expression of p53R2, a gene which is homologous with the R2 regulatory subunit of ribonucleotide reductase (RNR) (Nakano et al. 2000). p53R2 functions in a non-specific manner to increase the pool of free dNTPs when the need for repair arises. Although p53R2 and R2 are similar, they differ in their N-terminal amino acid sequence and regulation. p53R2 is induced by p53 and p73, while R2 synthesis occurs during S phase. The p53R2 and R1 complex functions as an active RNR (Guittet et al. 2001). p53 upregulates two very important proteins along the MMR pathway: human MutS homologue 2 (hMSH2) and proliferating cell nuclear antigen (PCNA) (Scherer et al. 2000, Xu and Morris 1999). Mutations of hMSH2 result in hereditary nonpolyposis colorectal cancer, a colorectal cancer syndrome. hMSH2 functions in mismatch recognition and binds mismatched bases (Lamers et al. 2000). PCNA, a cofactor for DNA polymerase δ, is another p53 target gene and has been shown to interact with hMSH2 to facilitate hMSH2 transfer to mismatched bases (Flores-Rozas et al. 2000).

Alterations in the ras gene have been identified in several neoplasias that have been chemically induced in rodents. Mutations of the ras gene exist in about 20% of human neoplasias located in the colon, breast, lung, and bladder (Pritchard et al. 2003). Analysis of the ras gene isolated from the DNA of these neoplasias reveals that changes in the sequence of nucleotides correspond to the places where carcinogens interact with DNA. Each chemical compound creates its own unique fingerprint on DNA (Robbins and Cotran 2005).

Some authors classify the genes involved in carcinogenesis as caretaker and gatekeeper (Kinzler and Vogelstein 1997, Lai and Shields 1999). This classification is based on their involvement in maintaining genome integrity and DNA repair, respectively (Lai and Shields 1999). The caretakers are responsible for maintenance of genome stability. Mutations in the caretaker genes, which are considered to be typical tumour suppressors, compromise genome stability and, more specifically, increase the probability of mutation in the gatekeepers which include both tumours suppressor genes and oncogenes (Vogelstein and Kinzler 2004, Blagosklonny 2005). Gatekeeper genes regulate neoplasic development by inhibiting its growth or killing it (Kinzler and Vogelstein 1997). In contrast, inactivity by caretaker genes does not support the starting phase of a neoplasia, instead favouring the genetic instability which results in an increase in mutations across all genes, including the gatekeeper. A neoplasia initiated by the inactivity of a gatekeeper gene can progress quickly as a consequence of its effect on genes that directly control cell death (Kinzler and Volgestein 1997).

EVALUATION OF CARCINOGENICITY

A major change in the field of carcinogenesis research has occurred over the last two decades with the development of analytical methods that are sensitive enough to detect background damage to DNA in healthy humans (Sharma and Farmer 2004). The control of responses to

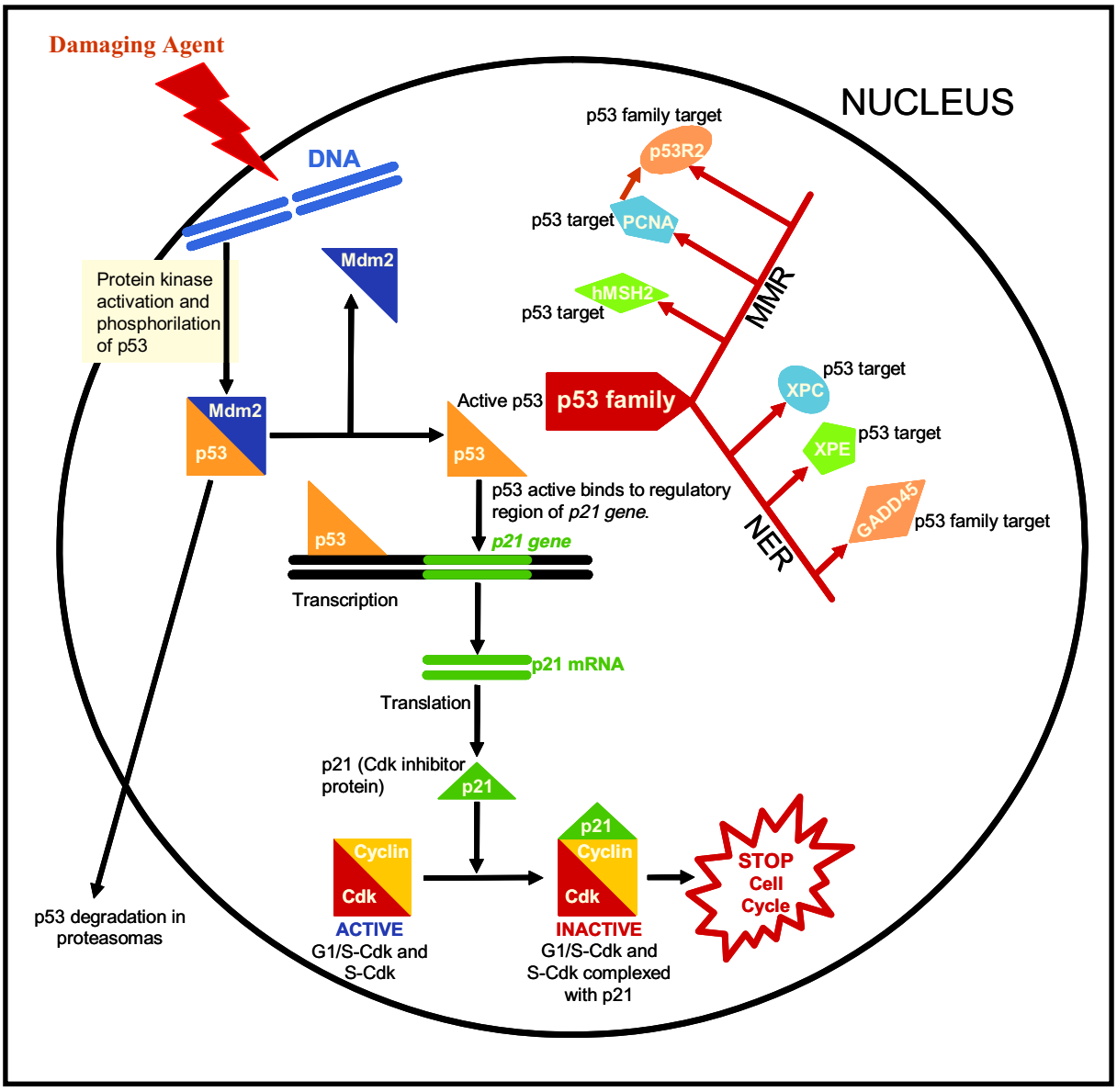

Fig. 7 – DNA repair mediated by p53 family target genes. Some mutations, which are linked to cancer, appear to involve the failure of one or many of a given cell's repair systems. One example of such an error involves DNA mismatch repair (MMR). After DNA copies itself, proteins from mismatch repair genes act as proofreaders to identify and correct mismatches. If a loss or mutation occurs in the mismatch repair genes, sporadic mutations are more likely to accumulate. Other errors in repair may involve bases or even whole nucleotides being incorrectly cut out (Nucleotide-excision repair-NER) as repair proteins try to fix DNA after bulky molecules, such as the carcinogens in cigarettes, have attached themselves. This is classed as faulty excision repair. Any of these mistakes (and others not appearing in the figure shown) may enable mutations to persist, be copied, and eventually contribute to cancer development. Both, MMR and NER, are repair processes mediated by p53 family proteins. p53 is a transcription factor whose activity is regulated by phosphorylation. The function of p53 is to prevent the cell from progressing through the cell cycle if DNA damage is found. It may do this in variety of ways; from holding the cell at a checkpoint until repairs can be made, to causing the cell to enter apoptosis if the damage cannot be repaired. The critical role of p53 is evidenced by the fact that it is mutated in a very large proportion of tumours from nearly every possible source.

carcinogenesis through the application of several chemical, biochemical and biological techniques facilitates the identification of those basic mechanisms involved in neoplasic development (King et al. 1995, Maronpot and Boorman 1996). Experimental assays with laboratory animals, epidemiological studies and quick tests enable the identification of carcinogenic compounds, the dissection of many aspects of carcinogenesis, and the establishment of effective strategies to prevent the cancer which results from exposure to chemicals (Grisham et al. 1984, Butterworth et al. 1992, Maronpot and Boorman 1996, Airoldi et al. 1999).

In vitro Assays of Cell Transformation

In vitro models are used to study the molecular mechanisms inherent to the neoplasic transformation of normal cells (Guengerich 2000, Achanzar et al. 2002). These assays use prokaryotic and human cells, have differing levels of complexity, and can overcome the ethical aspects related to animal experimentation (Masters 2000).

In 1970, a number of laboratory tests were developed to evaluate the mutagenic power of different chemical compounds, with the Ames test gaining particular distinction. This test semi-quantitatively evaluates a chemical's ability to induce mutations in *Salmonella tiphymurium* in a culture medium improved with microsomatic enzymes (Ames 1984). Between 70 and 90% of known chemical carcinogens show positive results on the Ames test. Most mutagenic chemicals in vitro are carcinogenic in vivo. Due to the high correlation that exists between mutagenecity and carcinogenicity, the Ames test is frequently used to evaluate the carcinogenic potential of chemicals. However, substances such as nitrosamines and beryllium do not strongly correspond to their results in the Ames test (Gonzalez 2001, Payne and Kemp 2003). It has been estimated that at least one hundred methods of in vitro testing the carcinogenic power of a compound have appeared over the last two decades.

Some scientists have questioned whether cells in culture maintain their bioactivation and detoxification mechanisms (Masters 2000, Gutiérrez and Salsamendi 2001). To validate the results obtained from these assays it is important to check if these results occur under physiological conditions considered as normal. To overcome the advantages of these methods, and those previously mentioned regarding in vivo assays, new methods were developed using human tissues and biological fluids to obtain specific biomarkers, which combined with the epidemiological studies gave results that are more reliable. These experiments are labelled as the molecular epidemiology of cancer or molecular dosimetry (Bondy 2004, Yang and Schlueter 2005).

In vivo Assays of Carcinogenesis

Experimental models with animals have been used successfully for a number of decades. They have enabled us to understand diseases, to discover etiological factors and to test many treatments (Maronpot and Boorman 1996). There are innumerable anatomic, physiological and biochemical resemblances between rodents and humans that justify their use in carcinogenicity testing (Maronpot and Boorman 1996, Balmain and Harris 2000). Results obtained from these studies permit the identification of the harmful carcinogenic compounds in the absence of real and credible human references and protect the public health (Huff 1992).

Current strategies to identify the carcinogenic potentiality of certain compounds include experimental protocols lasting a minimum of two years (Payne and Kemp 2003). These can stretch from 5 to 7 years if we take into account the posterior analysis of the results obtained via the different methods (Tennant et al. 1999). These assay groups of males and females, of mice and rats, are exposed to two or three doses of the agent being tested while a non-exposed (control) group is also used (Weisburger 1999). The experiment has a previously established duration and the animals that survive are sacrificed at the end of the experiment (van Leeuwen and Zonneveld 2001, Pitot 2001, Payne and Kemp 2003).

Animals are examined post-mortem in order to evaluate the incidence of neoplasic development and other pathological changes. Statistical analysis is used to evaluate if the neoplasic incidence is significantly different from the control group (Ito et al. 1992, Lutz 1998, Camargo et al. 1999, Tennant et al. 1999, Payne and Kemp 2003). On the cases in which the control animals do not show neoplasias, the results are considered significant if 10% of the animals exposed to the carcinogen develop neoplasias (Pitot 2001).

Carcinogenic assays on rodents identify potential

carcinogens for humans. Achieving a positive result on a conventional essay indicates that there exists only a potential danger. Its meaning for human health will depend on other factors, some of which require additional studies (Maronpot and Boorman 1996). The extrapolation of results obtained via experimental work with rodents is contested by the following arguments (Gaylor and Chen 1986, Huff 1992, Tennant et al. 1995, Haseman et al. 2001, Waddell 2002):

a) It has not been confirmed if rodent models are representative of carcinogenesis in humans.
b) The studies are too long.
c) The doses are too high and may cause a proliferative response in normal cells.
d) Many of the effects observed in animals have little importance for man.
e) The protective effects of the organism, metabolic detoxification, and DNA repair cannot be taken into account once they are overwhelmed by exposure to high doses.
f) Synergic effects are not taken into account with other chemical compounds.

Based on data accumulated from experiments in recent years, and according to Gutiérrez and Salsamendi (2001), they provide the following factors which favour these assays:

a) All substances that revealed carcinogenic activity in humans, apart from rare exceptions, are also positive in rodent assays.
b) Although many chemical carcinogens for animals do not cause cancer in humans, many of human carcinogens were discovered from assays in animals such as: aflotoxins, diethylstilbestrol or vinyl chloride.

Molecular biology has provided new models with which to study carcinogenesis with the development of transgenic and knockout rodents. Some models have mutations in the ras proto-oncogenes and in the p53-suppressor gene (Sills et al. 2001, Pitot 2001). Animal models deficient in p53 protein and ras genes are more sensitive to the identification of genotoxic carcinogens (Sills et al. 2001). According to Pritchard et al. (2003), the utilization of transgenic models to identify carcinogenic compounds has the following advantages:

a) Tumours developed more quickly.
b) The assays are shorter, with a duration of 24 to 26 weeks.
c) Fewer animals are used.
d) Through genetic modification, it is possible to identify those mechanisms associated with neoplasic development.

Although these models are promising, they also have limitations because they can exhibit metabolic alterations, which are not consistently relevant to carcinogenesis. In addition, mutated genes can influence the nature of neoplasia that is developed, increasing the difficulty of measuring the response in humans (Pritchard et al. 2003).

It is necessary to pay attention to the analysis of the results, because there is evidence which indicates that carcinogens can act through specific mechanisms. The premise that those carcinogenic compounds experimentally tested are harmful for man is not always valid (Swenberg et al. 1992, Cohen and Lawson 1995). The results obtained using rodents act as back-up against any false negatives obtained through in vitro researches and can be used to prevent, or reduce, human exposure to a suspected carcinogen (Payne and Kemp 2003).

Epidemiological Studies

Epidemiological studies provide a great deal of information about exposure to those chemicals present in food, the environment and at work, but are limited as far as the identification of etiological factors are concerned, especially in cases where neoplasic development results from the interaction of multiple agents (Garner 1998, Tennant 1998, Weinstein 1991). Epidemiological studies are retrospective and unless a large number of individuals are studied their sensitivity is reduced (Weinstein 1988, Tennant 1998).

Epidemiological techniques have been useful for identifying exposure to high carcinogenic concentrations. Yet, it is difficult to understand the individual contribution of a certain chemical within a complex situa-

tion like environmental contamination. Carrying out epidemiological studies of a scientific nature is difficult for several reasons (Farmer 1994, Tennant 1998):

a) The difficulty in evaluating external and internal exposition to chemicals.

b) The impossibility of simultaneously controlling exposure to other chemicals, and analysing the influence of those environmental and physiological factors that influence the evolution of the disease.

c) The latency period between initial exposure and cancer development.

Only in some cases, such as with tobacco smoke, does the epidemiological evidence of cause and effect be held beyond any doubt (Gutiérrez and Salsamendi 2001).

Other Methods

The carcinogenic influence of a substance can be determined using computer programmes that thoroughly simulate man's physiological and metabolic procedures and relate them to the molecular configuration of the substance being studied (Loew et al. 1985). These chemical properties are related to the molecular structure of chemical, physical, and toxicological properties (Barratt and Rodford 2001, Feng et al. 2003).

Statistical learning methods have recently been explored as a new approach for genotoxicity prediction without any restrictions on the features of structures or types of molecules. Instead of focusing on specific structural features or a particular group of related molecules, these methods classify molecules into genotoxic positive or non-genotoxic agents based on their general structural and physicochemical properties, regardless of their structural and chemical types (Li et al. 2005).

Other available tests concern the use of protozoa cultures and the chorioallantoic membrane. The ciliated protozoan *Tetrahymena pyriformis* may be used in bioassays to evaluate the cytotoxic impact of many chemical compounds (Bonnet et al. 2003). The chicken chorioallantoic membrane assay is used to study angiogenesis during tumour growth (Tufan and Satiroglu-Tufan 2005).

CONCLUSIONS

In summary, our objectives for this article were to review the current information available on chemical carcinogenesis. Chemical carcinogenesis is a multistage and multicausal process in which normal cells become first initiated, then malignant and invasive. Each of these stages is exceedingly complex in itself. The acquisition of the capacity to survive and grow independently from other cells represents a crucial event in the mechanism of cancer development. Most of the morphological, biochemical and genetic changes currently observed should be considered as the expression of the adaptation of neoplasic cells to survive in a familiar but hostile environment. The prediction of chemical carcinogenicity is of great importance to human risk assessment.

ACKNOWLEDGMENTS

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RESUMO

A sociedade obtém numerosos benefícios da utilização de compostos químicos. A aplicação dos pesticidas, por exemplo, permitiu obter alimento em quantidade suficiente para satisfazer as necessidades alimentares de milhões de pessoas, condição relacionada com o aumento da esperança de vida. Os benefícios estão, por vezes associados a desvantagens, os efeitos resultantes da exposição a compostos químicos enquadram-se entre a morte imediata e um longo processo de carcinogênese química. A carcinogênese química inclui três etapas definidas como iniciação, promoção e progressão. Cada uma delas caracteriza-se por transformações morfológicas e bioquímicas, e resulta de alterações genéticas e/ou epigenéticas. No grupo das alterações genéticas incluem-se mutações nos genes que controlam a proliferação celular, a morte celular e a reparação do DNA – i.e. mutações nos proto-oncogenes e genes supressores de tumor. Os fatores epigenéticos, também considerados como caracteres não genéticos, podem contribuir para a carcinogênese por mecanismos de silenciamento gênico. A utilização de diferentes metodologias possibilita o reconhecimento e a compreensão dos mecanismos básicos envolvidos no desenvolvimento do cancro. Ensaios experimentais com animais de laboratório, estudos epidemiológicos e alguns testes

rápidos permitem identificar compostos carcinogênicos, analisar os eventos envolvidos na carcinogênese e estabelecer estratégias para prevenir a exposição a estes agentes.

Palavras-chave: etapas da carcinogênese, avaliação de carcinogeneicidade, carcinogênicos químicos, carcinogênese química.

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