CHAPTER 11.

Mechanisms of carcinogenesis: from initiation and promotion to the hallmarks

Bernard W. Stewart

PART 2
CHAPTER 11

Introduction

For many decades, a corollary to the contemporary understanding of the nature of cancer and of carcinogenesis has been the recognition of causative agents. Since the 1950s, many agents that contribute to the development of cancer have been categorized as initiators or promoters, on the basis of studies of chemical carcinogenesis in mouse skin (Berenblum and Shubik, 1947).

Cancer was described with reference to causative agents. Thus, a 1970s pathology text (Cappell and Anderson, 1974) introduced

malignancy by describing a tumour as "an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the surrounding tissue, and that continues to grow in the same excessive manner after cessation of the stimulus that caused it". According to the same textbook, development of tumours of the skin, the alimentary canal, or the respiratory tract was to be expected among individuals exposed "to various noxious agents in the environment".

Causation of cancer in humans or animals by certain chemicals, radiation, and biological agents was recognized by early in the 20th century.

The types of biological agents and of radiation now recognized by IARC as *carcinogenic to humans* (Group 1) are few compared with the number of chemicals in this category (IARC 2012a, b, c, d, e, f); there is a much larger number of chemicals for which at least some evidence of carcinogenicity is available (see Volumes 1–105 of the *IARC Monographs*, available from http://publications. iarc.fr).

Research has established how many carcinogenic chemicals cause, or are likely to cause, malignant transformation, but the biological processes involved are diverse, and

Table 11.1. A selection of proposals for the categorization of chemical carcinogens[a]

Mode of action	Exposure context	Chemistry	Human relevance of bioassay data	Agent type
Genotoxic	**Tobacco smoke**	**PAHs**	**DNA binding**	**Atmospheric pollutants**
Direct-acting	**Alcoholic beverages**	***N*-nitroso compounds**	PPARα activation	**Pesticides**
Pro-carcinogen	**Occupation**	**Aromatic amines**	α_{2u}-Globulin nephropathy	Organic solvents
Inorganic carcinogen	**Pollution**	**Halogenated organic compounds**	Urinary tract calculi	Endocrine disruptors
Non-genotoxic	**Diet**	**Naturally occurring compounds**		Disinfection by-products
Solid-state carcinogen	**Pharmaceutical drugs**	**Inorganic compounds**		**Pharmacological steroids**
Hormone	**Exogenous hormones**			
Immunosuppressant				
Promoter				

PAHs, polycyclic aromatic hydrocarbons; PPAR, peroxisome proliferator-activated receptor.

[a] Knowledge about chemical carcinogens is presented from a variety of perspectives apart from that of mechanism of action. The listings indicate those used in particular publications (e.g. Searle, 1984; Tomatis et al., 1990; Vainio et al., 1992; Vainio and Hietanen, 2003; Hsu and Stedeford, 2010) as ways of ordering data, as indicated by chapter headings in many cases, and are not necessarily comprehensive. Categories shown in **bold** involve or include at least one Volume 100 (Group 1) agent.

there is no generally accepted mechanistic basis for classifying chemical carcinogens (Loeb and Harris, 2008), beyond categorization according to genotoxicity (Weisburger and Williams, 1981). There is no single comprehensive basis for categorization; chemical carcinogens are sometimes ordered according to the context in which information is presented, with genotoxicity ordered according to mutational signatures, or agents categorized in relation to differing classes of receptors. There have been many proposals for the categorization of chemical carcinogens according to various criteria. A selection of these is shown in Table 11.1; others include the categorization of chemical carcinogens on the basis of the organ affected (Warshawsky and Landolph, 2006).

Currently, the most widely recognized description of the nature of cancer is that presented by Hanahan and Weinberg in two reviews – published more than a decade apart – that identify the "hallmarks" of cancer (Hanahan and Weinberg, 2000, 2011). These papers have been so influential that others refer to "the hallmarks" without further qualification, for example in the title of a recent perspective on tumour metabolism (Cantor and Sabatini, 2012).

Since 2000, about 200 cancer research papers with a title including "hallmark" or "hallmarks" have been published. These papers typically describe signal transduction pathways and their therapeutic implications. Although the characterization by Hanahan and Weinberg (2011) of the hallmarks of cancer did not refer to chemical carcinogens or causative agents in general, recently the hallmarks have been used to characterize chemical carcinogens (Kleinstreuer et al., 2013).

These considerations give rise to two questions: (i) whether previously used mechanism-based descriptions of chemical carcinogens may be recast in relation to the hallmarks; and (ii) whether, and to what extent, the hallmarks provide opportunities

to characterize agents apart from currently known carcinogens as contributing to the development of cancer. Both of these matters are addressed in this chapter.

Multistage carcinogenesis

Exogenous agents

The widely accepted paradigm of carcinogenesis as involving a multistage process is generally recognized to have been developed from the two-stage model of carcinogenesis in mouse skin (Berenblum and Shubik, 1947), which typically involves a polycyclic aromatic hydrocarbon (PAH) and a phorbol ester (identified as the active agent in the irritant croton oil). Because tumorigenesis in animals is amenable to histological examination at all stages, morphological criteria can be used to characterize the process. With the production of malignant tumours as the end-point, two-stage or multistage carcinogenesis was readily described in various organ sites in animals, including the liver and the bladder (Slaga et al., 1978).

Thus, in relation to hepatocarcinogenesis, agents such as phenobarbital, dichlorodiphenyltrichloroethane, polychlorinated biphenyls, butylated hydroxytoluene, and estradiol benzoate were identified as promoters (Dohi et al., 1996). The relevant experimental observations, in addition to indicating the possible risk to humans presented by the relevant chemicals, also led to the contemporary understanding of the nature of malignancy itself. That understanding was based on the identification of particular abnormal cell populations, specifically including chemically induced hyperplastic nodules in rat liver (Farber, 1973).

Morphological and genetic changes

Within 20 years of the publications cited above, the identification of multistage carcinogenesis with particular carcinogens or other exogenous agents had become irrelevant to an understanding of cancer development. Over the same decades, the context in which carcinogenesis was best understood changed from rodents to humans. Critical to this transition was the identification of multistage carcinogenesis with alterations in gene structure or expression rather than with the impact of exogenous agents.

A key development was the correlation by Vogelstein et al. (1988) of morphological change during the development of colon cancer in humans with particular genetic change. The concept was applicable to all tumour types. Thus, in a diagram illustrating multistage carcinogenesis with respect to human lung cancer, Harris (1992) made no reference to any particular exogenous agents as mediating specific stages in tumorigenesis, and showed the transitions between stages as being mediated by alterations in the structure or expression of oncogenes and tumour suppressor genes.

Oncogenes and tumour suppressor genes mediate altered proliferative activity in a positive and negative sense, respectively. Classically, increased proliferative activity due to oncogene expression accounted for the transformation of NIH 3T3 cells by DNA isolated from tumours and not by DNA from normal tissue (Shih et al., 1981). Oncogene activation (e.g. mutation of *Ras*) has shown that although binding of many chemical carcinogens to diverse

biological macromolecules had been variously demonstrated over decades, carcinogen adducts in DNA were crucial.

Alkylation of DNA by *N*-nitroso compounds was shown by Magee and Farber (1962), with tumorigenesis attributable to the pro-mutagenic O^6-methylguanine product, which mispairs with thymine. In rats, activation of H-*Ras* in mammary gland tumours induced by *N*-methyl-*N'*-nitrosourea was correlated with H-*Ras* mutation at codons 12, 13, and 61 (Sukumar et al., 1983). However, although this insight had been gained, it was clear that the etiology of some types of cancer, such as breast cancer in humans, did not primarily involve alkylating agents. Thus, in human cancer *RAS* activation is a relevant genetic change in tumour tissue, without reference to exogenous agents (Bos et al., 1987).

Although the concept of multistage carcinogenesis was established through the use of exogenous agents that target particular organ sites in animals, by 1990 multistage carcinogenesis was primarily identified with altered structure or expression of genes associated with cell proliferation, specifically as described in human tumours. However, the focus of that research has not involved the specification of genetic change over time in a manner that might account for the emergence of a metastatic cell population from within normal tissue. Rather, the relevant research has involved the identification of disordered signal transduction pathways, with a view to developing targeted therapies. The archetype of such research is that establishing the transforming role of the tyrosine kinase BCR-ABL in chronic myeloid leukaemia, and its inhibition

– to the great benefit of patients – by the low-molecular-weight inhibitor STI-571 (imatinib) (Bilanges and Stokoe, 2007; Rosa et al., 2008).

Molecular changes

Among a series of reviews marking the publication of the 100th volume of the journal *Cell*, Hanahan and Weinberg (2000) delineated the very wide (even then) spectrum of studies addressing the genetics of cancer by reference to phenotype. Six characteristics of how cancer cells behave could be identified in relation to particular genes or classes of genes. The phenotypic characteristics were: uncontrolled proliferative activity (Hall, 1984), tumour growth attributable to familial risk (Hussain and Harris, 1998), survival of cancer cells (Vaux et al., 1988), immortalization of cancer cells (Sedivy, 1998), growth of blood vessels in tumours (angiogenesis) (Cavallaro and Christofori, 2000), and metastatic growth (Webb and Vande Woude, 2000). Accordingly, the hallmarks of cancer were initially identified as follows:

• self-sufficiency in growth signals;

• insensitivity to anti-growth signals;

• evasion of apoptosis;

• sustained angiogenesis;

• limitless replicative potential; and

• tissue invasion and metastasis.

The 2000 "hallmarks" review was concerned primarily with the characterization of the genes and associated signal transduction pathways that mediate these respective activities in malignant cells and tumours. In that paper, hypothetical patterns of multistage carcinogenesis were illustrated by a linear arrangement of the pictograms for the hallmarks, without reference to any morphological criteria. From that diagram, it can be inferred that some hallmarks – such as self-sufficiency in growth signals –

emerge early, whereas others – sustained angiogenesis, and tissue invasion and metastasis – are seen later.

Although hallmarks such as sustained angiogenesis and metastasis involve morphological change, all of the hallmarks were identified with reference to changes in gene expression and not by reference to, or necessarily in correlation with, a change in morphology. Diversity between tumour types and within a given tumour type was noted, and no reference was made to any particular type of neoplasm for illustrative purposes.

In such a description of the manifestation of essential alterations that collectively characterize malignant growth, there is no requirement to identify exogenous agents as acting on normal or premalignant cells to cause the change. The focus is on the nature of tumours and how they may be distinguished from relevant normal tissue. Finally, Hanahan and Weinberg (2000) identified an enabling characteristic: genomic instability, which is equated with increased mutability evident during the process of tumour progression (Loeb, 1994).

A decade on: "the next generation"

In 2011, Hanahan and Weinberg provided a new assessment of the hallmarks (Hanahan and Weinberg, 2011). They commented, "The past decade has witnessed remarkable progress towards understanding the mechanistic underpinnings of each hallmark." One indication of progress is that the original hallmarks were rebadged as follows:

• sustaining proliferative signalling;
• evading growth suppressors;
• resisting cell death;
• inducing angiogenesis;

• enabling replicative immortality; and

• activating invasion and metastasis.

It is notable that, in almost every instance, the hallmark is not the name of a phenotype but refers to a dynamic process. Consistent with this perception, the authors wrote, "The hallmarks of cancer comprise six biological capabilities acquired during the multistep development of human tumours. The hallmarks constitute an organizing principle for rationalizing the complexities of neoplastic disease."

In addition, a decade of progress had enabled the specification of two "emerging hallmarks":
• deregulating cellular energetics; and
• evading immune destruction.

The enabling characteristic identified in 2000 as "genomic instability" was described in 2011 as "genomic instability and mutation", and a second enabling characteristic was identified as "tumour-promoting inflammation". Superficially, such reference to mutation and to promotion might be seen as implying, if not specifying, the roles that DNA-damaging and proliferation-inducing agents have in carcinogenesis. However, this is not the case.

In this context, "mutation" refers to an acceleration of the accumulation of mutations, due to, among other things, defects in the DNA maintenance machinery (Kinzler and Vogelstein, 1997). As a result, mutation occurs more readily, irrespective of whether it is mediated by exogenous or endogenous agents. Accordingly, DNA adducts, strand breakage, and related phenomena are not to be identified with this enabling characteristic and do not account for, or are not properly identified with, a particular hallmark. Mutation, in the context of

carcinogenesis, identifies a mechanism whereby a chemical carcinogen may cause the emergence of any of the hallmarks, and almost certainly of several of them, or perhaps of all of them. The enabling characteristic "genomic instability and mutation" renders such outcomes more likely (Wang et al., 2012), rather than referring to the mechanism through which the change occurs.

The identification of "tumour-promoting inflammation" as the second enabling characteristic recognizes that inflammation causes the emergence of several of the hallmarks, including sustaining proliferative signalling and inducing angiogenesis. In their discussion of this enabling characteristic, Hanahan and Weinberg (2011) were concerned primarily with cellular infiltration by cells of both the innate and the adaptive arms of the immune response. They made scant, if any, reference to exogenous agents provoking an inflammatory response.

From a broad perspective, reference to the multistep development of human tumours provides a way to consider the particular impact of carcinogens and other exogenous agents that may contribute to cancer development. However, in identifying the hallmarks, Hanahan and Weinberg did not pursue this matter.

Identifying mechanisms of carcinogenesis

As mentioned above, chemical carcinogens have been categorized primarily with reference to whether they exhibit genotoxicity. This mechanistic distinction began with many then-known carcinogens being identified as mutagens in vitro by use of particular bacterial strains and after metabolic activation (the Ames test) (McCann et al., 1975). The term "genotoxic" indicated, among other things, that the covalent binding of a carcinogen adduct to DNA, when evident, might account for carcinogenesis. Thus, Weisburger and Williams (1981) categorized carcinogens primarily on the basis of genotoxicity. Research over the subsequent 30 years did not alter that approach (Hsu and Stedeford, 2010).

The multiplicity of agents and the relatively limited understanding of their respective mechanisms of action have precluded the adoption of a scheme for categorizing carcinogens beyond the consideration of genotoxicity. Arguably, until the present IARC Scientific Publication, the most authoritative assessment on how carcinogens act was the 35-page consensus report in the publication *Mechanisms of Carcinogenesis in Risk Identification* (Vainio et al., 1992); this was the agreed position of a Working Group of more than 40 scientists in 1991. The consensus report did not centre on a scheme for classifying carcinogens according to their mechanism of action.

Across decades, commentaries on chemical carcinogens (Van Duuren, 1980; Pitot, 1990; Xue and Warshawsky, 2006; Cohen and Arnold, 2011) have not been based on any generally agreed categorization according to mechanism of action. Rather, the common theme has been the enumeration of biological parameters that may determine whether tumours develop in response to carcinogens in general.

Genotoxicity: progress and problems

Multiple indicators of genotoxicity have been recognized and categorized as involving data generated either in vitro or in vivo (Montesano et al., 1976). In vitro test systems include bacterial, mammalian, and other cells, with weight being given to the extent to which the test system has been "validated", as summarized by sensitivity and specificity in relation to known carcinogens and non-carcinogens. In vivo indicators of genotoxicity include, among others, (i) metabolism of a chemical to produce reactive, typically electrophilic, intermediates, which are the source of adducts bound to DNA and other macromolecules, and (ii) evidence of subsequent DNA repair and/or mutation.

This description of indicators of genotoxicity also summarizes the relevant mechanism of chemical carcinogenesis as currently understood (Cohen and Arnold, 2011). Thus, carcinogen metabolism and DNA repair processes have been used to identify candidate genes for lung cancer susceptibility studies (Yokota et al., 2010). Compared with the relatively modest number of genes that account for the absorption, metabolism, and elimination of a carcinogen, together with the repair of corresponding DNA adducts, the hallmarks (Hanahan and Weinberg, 2011) enable the specification of tens – if not hundreds – of genes whose expression contributes to the malignant phenotype.

At the single-gene level, mutation of *TP53*, specified with reference to particular transitions and transversions, is attributable to miscoding, which in turn is a consequence of DNA adduct formation from relevant carcinogens, including those in tobacco smoke (Soussi, 2011). The data provide evidence of particular exposures, but it remains unclear how tumorigenesis is enhanced by such mutation, beyond the consideration that a functional p53

Table 11.2. Chemicals cited by Ashby (1992) and Eastmond (2012) as examples of compounds with equivocal genotoxicity

Chemicals identified by Ashby (1992)	Chemicals identified by Eastmond (2012)
3-Amino-4-ethoxyacetanilide	Bromate
3-Amino-9-ethylcarbazole.HCl	Captan
Chlorinated paraffins	Carbon tetrachloride
CI Acid Orange 3	Chloroprene
CI Basic Red 9.HCl	Chromium(III)
Cinnamyl anthranilate	Chromium(VI)
1,2-Dibromo-3-chloropropane	1,3-Dichloro-2-propanol
di-Menthol	1,4-Dioxane
Methyldopa sesquihydrate	Ethylene glycol monobutyl ether
5-Nitroacenaphthene	Hydroquinone
4-Nitro-*o*-phenylenediamine	2-Nitrotoluene
Piperonyl butoxide	Trichloroacetic acid
Piperonyl sulfoxide	1,2,3-Trichloropropane
1,2-Propylene	
Sulfallate	

protein induces apoptosis, cell-cycle arrest, and senescence, and that these processes are compromised after *TP53* mutation (Bieging and Attardi, 2012). The hallmarks offer a broadened perspective as to signalling pathways that may be affected by mutation of *TP53* or any tumour suppressor gene.

In the first such determination made, genotoxic injury by tobacco smoke in one individual case of lung cancer accounted for 22 910 somatic base substitutions, of which 134 were in coding sequences (Pleasance et al., 2010). The role of tobacco smoke as a determinant of the genomic landscape of lung cancer has been confirmed, with an average mutation frequency in lung tumours from smokers of more than

10 times that in lung tumours from never-smokers (Govindan et al., 2012).

However, analysis of lung cancer genomics does not require immediate reference to smokers and never-smokers to present relevant data (Liu et al., 2012; Peifer et al., 2012). Moreover, the recognition of tobacco-induced genomic injury does not necessarily extend to other sites; for example, on the basis of individual genomic analysis, it is not possible to differentiate between cases of pancreatic cancer in smokers and in never-smokers (Wei et al., 2012).

More generally, although mutation of *TP53* is highly relevant to colorectal cancer, the impact of exogenous influences or causal factors on the development of this tumour type is

not evident from genomic analysis (Muzny et al., 2012). In short, the role of mutation as contributing to cancer development may be elucidated without reference to any genotoxic agent, even when the role of such an agent has been otherwise established.

Distinguishing genotoxic from non-genotoxic carcinogens

Even though molecular processes associated with genotoxicity are being defined in steadily greater detail, it is not always possible to immediately discriminate between individual chemicals on the basis of whether particular substances should be categorized as genotoxic. Difficulties are evident when relevant chemicals are considered on a case-by-case basis. More than

Table 11.3. Examples of categories of non-genotoxic carcinogens as variously proposed over more than three decades[a]

Weisburger and Williams (1981)	Weisburger (1989)	Marquardt (1999)	Hernández et al. (2009)	Benigni et al. (2013)
Solid-state carcinogens	Halogenated compounds	Cytotoxic carcinogens	Endocrine modifiers	Peroxisome proliferators
Hormones	Immunosuppressants	Tumour promoters	Receptor-mediated	Gap-junction inhibitors
Immunosuppressants	Hormones	Hormones	Non-receptor-mediated	DNA-methylating agents
Co-carcinogens	Solid-state materials	Immunosuppressants	Promoters	Agonists/antagonists of the aryl hydrocarbon receptor
Promoters	Certain hypolipidaemic carcinogens	Peroxisome proliferators	Tissue-specific toxicity and inflammation inducers	Oxidative stress inducers
	Phthalate ester plasticizers	Solid bodies or particles	Cytotoxic agents and immunosuppressants	Hormonal imbalance inducers
			Gap-junction inhibitors	

[a] Typically, the listings have been provided by the respective authors for illustrative purposes, without necessarily specifying an intent to be comprehensive.

20 years ago, Ashby (1992) reported on "practical examples of instances in which the term genotoxic is both needed and capable of having different meanings". Two decades later, Eastmond (2012) provided insight by summarizing data for another set of chemicals, different from those discussed by Ashby (Table 11.2).

Hence, there are some chemicals that are not readily categorized in relation to genotoxicity because, for example, they produce positive results when assessed by use of in vitro genotoxicity tests but after their administration to intact animals, they do not cause structural DNA damage or other manifestations of genotoxicity. As described by Eastmond (2012), apparently contradictory findings can be reconciled when, for different individual chemicals, account is taken of:

• the chemical properties of the agent, its metabolites, and/or its degradation products;

• the agent's metabolism and toxicokinetics;

• structural similarities to recognized mutagenic carcinogens;

• the origin of or mechanisms underlying the observed effects; and

• in vivo data, particularly in the target organ.

Eastmond (2012) illustrated each of these points with two or more examples.

Specifying genotoxicity is complex, as becomes evident when all available mechanistic data are identified, as occurs, for example, in *IARC Monographs* evaluations. In some instances, the totality of available mechanistic data may indicate that the categorization of a carcinogen as genotoxic is equivocal. There does not appear to be a context in which awareness of the hallmarks would provide an improved basis for identifying genotoxic carcinogens specifically.

Non-genotoxicity: multiple mechanisms and pathways

Regardless of any difficulty with particular agents as discussed in the previous section, the conceptual basis of genotoxicity is unequivocally focused on a particular pathway to malignant transformation. No such single focus is available for non-genotoxic carcinogens, as illustrated by the designation "epigenetic", which, although previously applied to these agents (Weisburger and Williams, 1981; Benigni et al., 2013), can no longer be unequivocally used in this context.

Epigenetic processes are relevant to both genotoxic and non-genotoxic agents (Pogribny et al., 2008), and epigenetic change may be determined by mutation (You and Jones, 2012). From a different perspective, when discussing non-genotoxic carcinogens, Meza et al. (2010) identified tobacco smoke and radon in

this context. Despite such ambiguity, 45 non-genotoxic carcinogens were recognized in 2009 among 371 agents classified by IARC in Group 1, Group 2A (*probably carcinogenic to humans*), and Group 2B (*possibly carcinogenic to humans*) (Hernández et al., 2009).

Grouping agents on the basis of a default criterion – i.e. that the agent is not genotoxic – implies uncertainty. The scope of uncertainty can be seen from differences between reports indicating categories of agents that are reasonably considered to be non-genotoxic carcinogens; Table 11.3 shows selected examples from 1981 to 2013.

Parameters used to identify non-genotoxic carcinogens include either the nature of the agent or some indicator of a putative mechanism of action. The terminology is far from definitive. Thus, while the term "promoter" may be used to identify a non-carcinogen that contributes to tumour development, tumour promotion may be identified with the action of many non-genotoxic carcinogens (Schulte-Hermann et al., 1999).

The role of receptors has long been recognized as key to the carcinogenicity of many non-genotoxic agents (Lucier, 1992) and underpins current commentaries (Klaunig, 2010). Relevant receptors include the aryl hydrocarbon receptor (AhR), the peroxisome proliferator-activated receptor (PPAR), and various hormone receptors.

Arguably, AhR is recognized mainly as mediating the carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD). However, as specified by Matsumura et al. (2009), apart from mediating toxic effects of some pollutants, AhR is involved in development, regulation of cell differentiation and cycling, hormonal and nutritional homeostasis, coordination of cellular stress responses (including inflammation and apoptosis), immune responses, and ageing. Therefore, it is difficult to identify AhR-mediated processes with a specific hallmark.

The adoption of a mechanistic approach to categorize non-genotoxic carcinogens leads to incongruities if definitive and exclusive specifications are sought. Thus, TCDD may be readily identified as a promoter (Ray and Swanson, 2009) while also being recognized as a complete carcinogen on the basis of bioassay and epidemiological data (Baan et al., 2009). Similarly, although PAHs can be identified with the genotoxicity of, for example, tobacco smoke, Puga et al. (2009) noted that exposure to toxic PAHs raises several toxic and carcinogenic responses in experimental animals and humans, mediated for the most part by AhR. Such apparent paradoxes indicate that although mechanistic categorization of many genotoxic carcinogens is definitive and exclusive, the same process applied to non-genotoxic agents may lead to outcomes determined by context. The relevant agents cannot be identified with a single path to malignancy.

The role of cell proliferation in relation to non-genotoxic agents also depends on the context (Preston-Martin et al., 1990; Marquardt, 1999). With respect to chemicals, the original focus was on mitogens, including peroxisome-proliferating carcinogens (Butterworth et al., 1992). This approach now identifies inflammation as contributing to cancer development, and auto-inflammatory disease and the impact of various cancer-causing infectious agents are equally recognized (Schetter et al., 2010). Cell proliferation in this context does not pertain to proliferation after toxic injury by genotoxic agents. Proliferative activity induced by genomic injury may be considered in relation to the pluripotent stem cells (Cohen and Arnold, 2011), further indicating how a characteristic – such as the hallmark "sustaining proliferative signalling" – cannot readily be assigned or restricted to a particular category of carcinogens.

Public health decision-making: the definitive consideration

This IARC Scientific Publication is based on evaluations made in Volume 100 of the *IARC Monographs*. Two broad issues are addressed: (i) the extent to which the occurrence and anatomical site of agent-attributable cancer in humans may be correlated with the occurrence and, where relevant, organ site of tumours in animals treated with the same agent; and (ii) whether known mechanisms of action of the carcinogenic agents in question, considered together with current knowledge of cancer etiology, reveal options for categorizing carcinogens, so as to better indicate the risk posed to humans by exposure.

These two considerations are intimately related. Thus, the occurrence or absence of tumours in rodents treated with particular agents may be wholly dependent on biological mechanisms operating, or not operating, in particular species. Until now, mechanistic assessment of carcinogens has not established a comprehensive basis for determining whether particular agents are capable of causing cancer in humans. This situation confirms that evaluations of the

IARC Monographs are appropriate for hazard identification, as distinct from any simple categorization of relevant agents. The fact that agents may be classified into Groups does not alter the need to make evaluations on a case-by-case basis.

The determination of whether a chemical induces cancer through a genotoxic mechanism frequently plays an important role in evaluating the risks associated with low exposures (Eastmond, 2012). For low levels of exposure to non-genotoxic carcinogens, there is expected to be a dose–response threshold for the carcinogenic effects; this does not apply to genotoxic carcinogens (Klaunig, 2010). Low-dose models of liver cancer induction in fish by genotoxic carcinogens indicate further levels of complexity (Williams, 2012), and ongoing controversy about non-monotonic responses means that such issues remain pertinent (Fagin, 2012). Mechanisms that underpin, for example, dose–response curves may become amenable to genomic and related analyses.

Systematic appraisal of mechanisms of carcinogenesis

Information about mechanisms of carcinogenesis for the Group 1 agents in the *IARC Monographs* is summarized in this Scientific Publication with initial reference to 24 mechanistic end-points, which were then merged into 10 key characteristics (see Chapter 10, by Smith). These end-points – which include DNA damage, changes in gene expression, receptor-mediated effects, and inhibition of gap junctional intercellular communication – have been adopted on the basis of their wide use to investigate mechanisms of carcinogenesis. Once the available data are ordered according to these end-points, it is evident that for many agents, simple categorization according to a single mechanism is not possible or appropriate.

An important consideration is the discrepancy between the extents to which end-points have been assessed. DNA damage and gene mutations have been studied most extensively, and agents for which there is unequivocal evidence of genotoxicity across in vitro and in vivo systems have rarely been studied in relation to, for example, epigenetic alterations. Epigenetic alterations have been described for estrogenic hormones (Imamura, 2011), arsenic (Jensen et al., 2008), and nickel (Costa et al., 2005), although each of these agents had also been characterized as causing DNA damage. Evidence of immunosuppression may have been considered as a singular mechanism of carcinogenesis, but while azathioprine can be characterized as immunosuppressive, this agent also causes DNA damage.

Having been adopted as described, the 10 key characteristics warrant review with reference to the hallmarks as cataloguing a broad biological basis for malignancy (Hanahan and Weinberg, 2011). One hallmark, "activating invasion and metastasis", is not recognized as a mechanistic end-point because few, if any, agents are identified primarily with metastatic growth, given that no such hazard needs to be established over and above carcinogenicity. Some hallmarks are singularly identified as mechanistic end-points or enabling characteristics, i.e. those corresponding to chronic inflammation, immune effects, cell death, and angiogenic effects. Arguably, the end-point "DNA repair alteration" correlates with the enabling characteristic "genomic instability and mutation". The end-points "alterations in telomere length" and "immortalization" address the hallmark "enabling replicative immortality".

It would appear that the hallmark "evading growth suppressors" corresponds to end-points identified by cell-cycle effects taken together with a subset within the end-point "gene mutations": the subset of mutation of tumour suppressor genes as distinguished from mutation of oncogenes or other genes. The default position would then be to identify "sustaining proliferative signalling" – arguably the premier hallmark – with the remaining end-points. However, reference to those end-points leads to the recognition that end-points such as "epigenetic alterations" are the means through which many, if not all, of the hallmarks may emerge.

Finally, "deregulating cellular energetics" remains as the hallmark not addressed through the characteristics identified, because this parameter has not been recognized in systematic efforts to characterize mechanisms of carcinogenesis. Overall, no particular insight appears to be gained by attempting to relate the 10 key characteristics with specific hallmarks.

Tobacco smoke, cancer of the lung, and the hallmarks

Generalizing across tumour types, genomic and comparable analyses are concerned little, if at all, with exogenous agents that mediate malignant transformation. Paradoxically, the first tumour genome documented was described with a total focus on mutations attributable to tobacco smoke (Pleasance et al., 2010). Although genomic analysis revealed

Fig. 11.1. Hallmarks of lung adenocarcinoma. Left: The prevalence of mutation or somatic copy number alterations of genes mapping to cancer hallmarks defined by Hanahan and Weinberg (2011) based on tumour specimens from a cohort of 183 patients of whom more than 85% had a history of smoking. Top right: Genes comprising the mutated genes in the hallmark "sustaining proliferative signalling" are shown. Bottom right: A proposed new hallmark of "epigenetic or RNA deregulation" is shown, depicted as above. Genes shown in grey are candidate lung adenocarcinoma genes identified in the study of Imielinski et al. (2012) that may additionally contribute to the hallmark. Reprinted from Imielinski et al. (2012), copyright 2012, with permission from Elsevier.
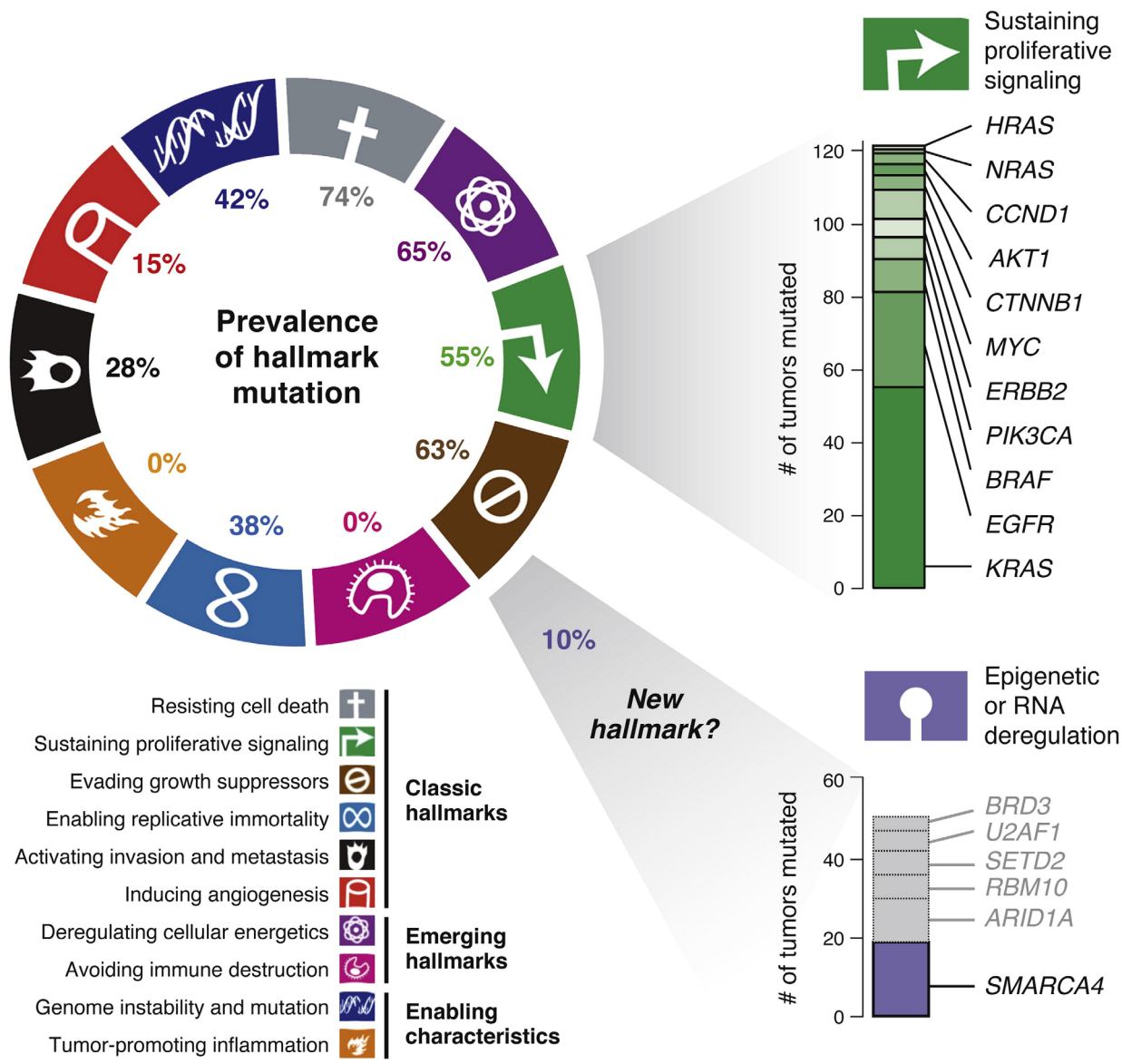
an average mutation frequency in lung tumours from smokers of more than 10 times that in lung tumours from never-smokers (Govindan et al., 2012), the genomic pattern of squamous cell lung cancer, established from 178 patients of whom 96% had a history of smoking, was presented with no overt reference to tobacco use (Hammerman et al., 2012).

The genomic profile of lung adenocarcinoma, involving a cohort of patients of whom more than 85% had a history of smoking, was presented with reference to the hallmarks, documenting the prevalence of the enabling characteristic "genomic instability and mutation" in 25 adenoma genes adopted as indicators (Imielinski et al., 2012). The findings were not presented with reference to smoking status but indicated markedly different fractions of mutation (Fig. 11.1), including 42% with respect to "genomic instability and mutation". This result indicates the requirement to distinguish between gene mutation being relevant to etiology, whether or not it is caused by an exogenous agent, and frequency of mutation being an indicator of genomic instability and thus a characteristic of malignancy. Also of note, only 6% of tumours had alterations assigned to all six original hallmarks.

Mutation of genes that mediate particular hallmarks and are attributable to, among other agents, N-nitroso derivatives of nicotine and related compounds, and PAHs, is to be expected. However, beyond lung cancer, there are only few references to genomic analyses that enable individual tumours attributable to smoking to be distinguished from others. Thus, genomic analysis did not reveal likely tobacco causation for particular pancreatic cancers (Biankin et al., 2012).

Possible inferences from hallmark-based studies

Any malignancy is expected to exhibit the hallmarks, whether it arises spontaneously or upon exposure to a carcinogen. Insight into mechanisms of carcinogenesis is gained by the demonstration of biological change, which may be aligned with a hallmark (He et al., 2014). The public health implications of such a discovery may apply to agents not recognized as carcinogenic but shown to be promoters and/or inducers of inflammation or angiogenesis. Nicotine is an example of such an agent (Cardinale et al., 2012; Schaal and Chellappan, 2014). In addition to its contribution to a better understanding of tobacco smoke carcinogenesis, this information about the properties of nicotine is relevant to appropriate regulation of electronic cigarettes (also known as electronic nicotine delivery systems) (Dutra and Glantz, 2014). Nicotine may contribute to cancer development, for example by stimulating angiogenesis, in a manner not likely to result in the compound being designated a carcinogen.

During the past 50 years, the understanding and use of the term "carcinogenesis" has changed from that involving a necessary reference to one or more exogenous carcinogens to that involving intracellular processes leading to malignant transformation, with no necessary or implied reference to exogenous agents. This understanding has recently included the description of random mutations arising from DNA replication in normal non-cancerous stem cells as accounting for sporadic disease (Tomasetti and Vogelstein, 2015). However, another recent development is the identification of different mutational landscapes between classes of K-ras-driven tumours, depending on whether

oncogene activation was achieved by genetic manipulation or after exposure to an alkylating N-nitroso compound (Westcott et al., 2015). Hence, genomic analysis may reveal distinct patterns of tumour-associated changes that are dependent on etiology and relevant to the full scope of tumour-associated signal transduction as identified by the hallmarks.

Apart from any mechanistic categorization of carcinogens in relation to particular hallmarks, the hallmarks do provide a basis for innovation. Genes identified from the perspective of each hallmark provide a basis on which to analyse both known carcinogens and agents of unknown status in that regard. An indication of agents worthy of attention may well be achieved by adding hallmark-related targets in the context of high-throughput screening assays, as described by Kavlock and colleagues (Kleinstreuer et al., 2013). The outcome may be the recognition of new classes of toxins that contribute to increased risk of cancer.

Summary

Cancer was once described with reference to causative agents, and multistage development of tumours was characterized through the impact of particular chemicals. Subsequently, multistage development of cancer was identified with morphological change being correlated with altered genetic makeup. The more recent description of eight hallmarks of malignancy is based not on morphology or on the impact of carcinogens but on changes in gene expression, sometimes mediated by mutation, and on selection for growth.

In parallel to this evolution of our understanding of cancer, no generally recognized mechanism-based scheme for classifying carcinogens

has evolved beyond categorization of chemical carcinogens according to genotoxicity. When appropriately studied, both genotoxic and non-genotoxic agents may mediate genetic and epigenetic change, variously resulting in emergence of the hallmarks, with the relevant processes being facilitated by genomic instability and inflammation. Enhancing –omics-based screening procedures to specifically include signal transduction pathways associated with particular hallmarks may provide new understanding of agent-related carcinogenesis.

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