CHAPTER 13.

Alterations in cell proliferation, cell death, or nutrient supply

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Introduction

Mechanistic data have been included in Volume 100 of the *IARC Monographs*, and they vary with the agent studied. These data are especially dependent on the type of study and the contemporary understanding of the state of the science at the time of publication of the study.

As an outcome of the two-part IARC Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis, a mechanistic database was assembled for the IARC Group 1 carcinogens (see Chapter 22, by Krewski et al.). These agents were examined with regard to 10 key characteristics – one or more of which are commonly exhibited by these agents – that can be used to identify and organize mechanistic information related to cancer induction (see Chapter 10, by Smith; see also Smith et al., 2016). One of these 10 mechanistic categories of data is a composite that includes information on the ability of a carcinogen to alter cell proliferation, cell death, or nutrient supply.

Alteration of cell proliferation is identified through assays that detect replicative DNA synthesis, 5-bromo-2′-deoxyuridine (BrdU) labelling, proliferating cell nuclear antigen (PCNA) labelling, and hyperplasia or the occurrence of multinucleated cells by light microscopy, and through analysis of some of these end-points by use of flow cytometry (Gray et al., 1986; Jayat and Ratinaud, 1993; Stacey and Hitomi, 2008; Irish and Doxie, 2014). Although for many of the human carcinogens Volume 100 of the *IARC Monographs* contains more descriptive data under this category, primarily as changes in cell proliferation, these changes are inherently related to alterations in cell signalling and/or cell-cycle control.

Many challenges are associated with the use of data described for alterations in cell proliferation, cell death, or nutrient supply to examine mechanistic and tumour site concordance between humans and experimental animals. Several key mechanistic characteristics can result in or arise from changes in cell signalling

(e.g. inflammation, genotoxicity, and epigenetic alterations) and can have both genetic and epigenetic origins.

There are other levels of interdependence between the key mechanistic characteristics. Inflammation, excessive oxidative stress, and genomic instability are related (see Chapter 17, by Kane). Mutagenesis may also underlie some epigenetic events that change cell signalling. For example, mutations in genes involved in the methylation of DNA, modification of histones, and binding of microRNAs to the genome or to other RNAs may initiate epigenetic changes (see Chapter 12, by DeMarini, and Chapter 20, by Rice and Herceg).

Cell signalling pathways that regulate cell proliferation are not independent of those associated with other key mechanistic characteristics of IARC Group 1 carcinogens. Many of the genes associated with cell proliferation are also linked with apoptosis, inflammation, and several pleiotropic responses. Dysregulation in the mitogen-activated protein kinase (MAPK) pathway affects most, if not all, processes involved in cancer (Dhillon et al., 2007). The extracellular signal-regulated kinase (ERK) pathway of the MAPK family is most commonly associated with regulation of cell proliferation (Reuter et al., 2010).

The ability to use this characteristic to evaluate both mechanistic and tumour site concordance is influenced by recent developments in cancer research, by the overarching issue of how carcinogens may express certain characteristics, and by the question as to the biological basis of the differences between species, strains, target organs, and target cells in cell signalling and cell-cycle control.

This chapter focuses on issues associated with the understanding and interpretation of available data for this key mechanistic characteristic.

Genetic drivers of cell proliferation and apoptosis: complex relationships and pleiotropic roles of cell signalling molecules

Cell signalling is a process whereby proteins or other chemical messengers activate receptors at the cell surface and then transmit signals inside the cell via membrane-to-nucleus pathways. In healthy adults, cell proliferation, cell differentiation, and cell death determine the size of the proliferating cell population in soft tissues, for example surface epithelia, mucosal lining cells of excretory ducts, columnar epithelia lining the gastrointestinal tract and uterus, transitional epithelium of the urinary tract, and bone marrow and haematopoietic cells. These proliferating cells replace dead cells throughout life. Pathological effects (e.g. injury resulting from hepatocellular necrosis or partial hepatectomy) and physiological conditions (e.g. estrogen-induced effects on the endometrium during the menstrual cycle) can involve stimulation of cell proliferation (Engström et al., 2015).

The relationships between cell signalling molecules and pathways that control cell proliferation and programmed cell death (apoptosis) are complex. Numerous enzymes and cell signalling pathways are modulated during apoptosis, and dysfunction of cell death pathways is associated with initiation and progression of tumorigenesis; the products of proto-oncogenes (genes that encode proteins that stimulate cell proliferation, inhibit apoptosis, or do both) include transcription factors, chromatin remodellers, growth factors, growth factor receptors, signal transducers, and apoptosis regulators (Narayanan et al., 2015). In response to mitogens, cell proliferation is triggered by increased translocation into the nucleus of ERK 1 and 2 (ERK1/2), the last proteins in the MAPK/ERK cascade. Activating mutations upstream or within the ERK1/2 cascade are present in several human cancers, but ERK1/2 activation also occurs in cancers without mutation of components of the cascade (Plotnikov et al., 2011).

Ras, a small upstream guanosine-5′-triphosphate (GTP)-binding protein in several signalling pathways, has two isoforms, H-Ras and K-Ras, with different potencies to activate the MAPK/ERK pathway; the *KRAS* gene is more frequently mutated in human cancer, which can result in constitutive activation (McCubrey et al., 2007). H-Ras has been implicated as contributing to the cancer phenotype, through evasion of anti-growth signalling, angiogenesis, genetic instability, tissue invasion and metastasis, tumour-promoting inflammation, and changes in the tumour microenvironment (Engström et al., 2015). K-Ras promotes metabolic reprogramming, activation of proliferative signalling pathways, glycolysis, reduction of oxidative metabolism in the tricarboxylic acid cycle, and channelling of glucose intermediates into anabolic pathways, such as the hexosamine biosynthesis pathway.

The tumour suppressor protein p63, which is activated by DNA damage, cellular stress, and oncogenic signal transduction, has pleiotropic anti-proliferative and metabolic effects that include metabolic cell-cycle arrest (Robey et al., 2015). Numerous pathways have been

identified to be involved in disruption of resistance to cell death, and p53 has been described as implicated in cell-cycle arrest, apoptosis, regulation of metabolism, DNA repair, and every pathway linked to these processes (Narayanan et al., 2015). Disruption of the MAPK cascades, which are central signalling pathways that regulate a wide variety of cellular processes, is associated with induction and progression of various diseases, including not only cancer but also diabetes, autoimmune disease, and developmental abnormalities (Plotnikov et al., 2011).

The role of gene activation in carcinogenesis is also complex and evolving. Activation of protein kinase C, which acts as a catalyst for several cellular functions that are related to cancer (e.g. cell survival, proliferation, apoptosis, and migration), has been thought to enable tumour development. However, protein kinase C isozymes have recently been reported to be suppressed in human cancers, possibly through loss of function that suppresses other oncogenic signals (Antal et al., 2015). The gene for an RNA-binding protein that is highly active in blood cancers (i.e. RNA-binding protein Musashi homolog 2) is not directly mutated in tumours, but its activation affects the ability of RNA to be translated into proteins (Wang et al., 2015), and consequently its role in cancer has not been identified through mutation or gene expression patterns.

Genetic variability in cell signalling between species, strains, and target organs

Genetic variability between species has been described in terms of their genomic content and the regulation and expression of their genes. Both the genetic code, which specifies trinucleotides that identify amino acids, and the regulatory code that determines how DNA sequences direct gene expression are highly conserved between species. However, species differ in the composition and the length of the DNA sequences that use this language in the regulatory regions of their genes (Nitta et al., 2015).

The Mouse ENCODE (Encyclopedia of DNA Elements) Consortium reported that the degree of conservation is high: the mouse genome is similar to the human genome in size, structure, and sequence composition, and more than 80% of mouse genes have human orthologues. The chromatin landscape in a cell lineage is relatively stable in both humans and mice, transcription factor networks are substantially more conserved, and both the human and mouse genomes are pervasively transcribed (Vierstra et al., 2014; Yue et al., 2014). The pattern of chromatin states (defined by histone modifications) and the large-scale chromatin domains are highly similar between mice and humans, but there is a divergence in the regulatory landscape that confers plasticity both between cell types and between species (Yue et al., 2014).

Organ-specific genes are more highly expressed than housekeeping genes (i.e. those present in all tissues), and the highest organ-specific gene expression is observed in the testes, brain, liver, muscle (cardiac and/or skeletal), and kidney (Lin et al., 2014). Comparisons of gene expression between human and murine tissues showed similarities in gene expression profiles at the tissue and organ level. However, there were greater similarities within each species for non-coding and conserved protein-coding genes, which are likely to mediate species differences (Lin et al., 2014).

Human-to-mouse transgenic experiments demonstrate recapitulation of human gene regulation in mice, even in the case of human genes that lack murine orthologues. However, for distinct biological pathways, the expression profiles of many mouse genes diverged from those of human orthologues (Yue et al., 2014). A core set of candidate regulatory sequences were conserved and display similar activity profiles in humans and mice: expression patterns for genes that encode proteins in the nuclear and intracellular organelle compartments, and genes involved in RNA processing, nucleic acid metabolism, chromatin organization, and other intracellular processes. However, less interspecies concordance was observed for genes involved with the extracellular matrix, cellular adhesion, signalling receptors, and immune responses (Yue et al., 2014).

Within orthologous mouse and human cell types, there is conservation across species of the global fraction of regulatory DNA sequences that encode recognition sites for each transcription factor (Vierstra et al., 2014). However, between humans and mice there is variation in regulatory regions that govern individual gene systems and the occupancy pattern of transcription factors, with extensive *cis*-regulatory "rewiring", mediated by elements that recognize transcription factors. Although they have a common language in regulation, active elements in one species may be reassigned to a different tissue in another species (Vierstra et al., 2014). Thus, differences in the regulation of gene expression and

cell signalling between species and tissues may affect mechanistic and tumour site concordance.

Variability in mutation targets and cell signalling across tissues and in tumours

Effects from activation of the MAPK/ERK pathway, such as cell growth, prevention of apoptosis, and cell-cycle arrest, depend on the cell lineage; for example, activation of this pathway is associated with proliferation and drug resistance in haematopoietic cells, but the activation is suppressed in some prostate cancer cell lines (McCubrey et al., 2007).

The difficulty in predicting cell-specific effects on cell signalling is also illustrated by differences in certain biological responses between histological subtypes of lung cancer, i.e. adenocarcinoma versus squamous cell carcinoma, in human patients and in chemically induced lung cancer in mouse models. In A/J mice treated with urethane to induce adenocarcinoma, or with *N*-nitrosotris-(2-chloroethyl)urea to induce squamous cell carcinoma, inhibition of vascular endothelial growth factor has the opposite effect in these two tumour subtypes (increased apoptosis vs increased proliferation) (Larrayoz et al., 2014).

In another example, gene expression profiling was poor at distinguishing histological subtype and cell type of origin for human breast cancer, but a mouse model could demonstrate the correct genetic lesion and cell type to model human disease, by confirming that the origin of *BRCA1* mutation-associated breast cancer is a luminal estrogen receptor (ER)-negative mammary epithelial progenitor (Molyneux and Smalley, 2011). However, different mouse models with the same K-*ras* mutations, one chemically induced and the other genetically engineered transgenic, produced tumours with different gene expression patterns (Westcott et al., 2015) and showed differences not only in tumour susceptibility but also in model-dependent signalling pathways. (See Chapter 19, by Caldwell et al., for a discussion of host susceptibility factors that influence tumour site concordance.)

As noted above, human tumours carry mutations in genes that encode components of cell signalling pathways associated with cell proliferation and cell death. High mutation frequencies have been associated with tumours induced by particular carcinogens or mixtures of carcinogens, for example melanoma induced by exposure to ultraviolet light and lung cancer induced by exposure to tobacco smoke (Lawrence et al., 2013).

However, cancer is not a disease of uniform origin, progression, or cell biology. Different types of cancer show variation in overall mutation rate, predominant mutation type, and distribution of mutations along the genome. Epigenetic patterns of chromatin accessibility, histone modification, gene expression, and DNA replication timing are also cell lineage-specific (Polak et al., 2015). A study of 173 cancer genomes from eight different types of cancer, representing a wide range of tissues of origin, carcinogenic mechanisms, and mutational signatures, showed that chromatin features of the cell type of origin, and not of matched cancer cell lines, were the best predictors of local frequency of somatic mutations. Mutation density was associated with epigenomic features, i.e. it was lower in areas of active chromatin and transcription (Polak et al., 2015).

To create a comprehensive catalogue of genes responsible for the initiation and progression of cancer, 27 types of cancer were studied through sequencing of matched tumour and normal tissue samples as part of the Cancer Genome Atlas and the International Cancer Genome Consortium (Lawrence et al., 2013). However, as sample sizes increase, the number of putative significant genes also increases and the risk of false positives resulting from tissue heterogeneity between cancer types in mutation type, distribution, and frequency is highly variable. Across the 27 types of cancer, the median frequency of non-synonymous mutations varied over more than three orders of magnitude; half of the variation in mutation frequency was associated with tissue type of origin. Within cancer types, patient-specific mutation frequencies also spanned three orders of magnitude. This mutational heterogeneity was strongly correlated with DNA replication timing and with transcriptional activity, i.e. it was higher in late-replicating DNA regions and lower in highly expressed genes. Higher mutation frequencies occurring in late-replicating genes may be responsible for potentially false-positive putative cancer genes, such as the olfactory receptor genes and some genes cited in association with lung cancer (Lawrence et al., 2013). Thus, the tissue of origin greatly affects mutation patterns and is linked to DNA replication timing and tissue-specific transcriptional activity.

Genomic sequencing of established tumours to study their causes has its limitations, because such an approach is unable to study the

evolution of the clones, the accumulation of mutations in normal somatic cells, the variability among individuals in driver mutation profiles, and the variability among cancer genes in clonal dynamics. In a study of 74 cancer genes in sun-exposed skin, i.e. a polyclonal quilt of mutations in key cancer genes consistent with damage from ultraviolet light, multiple cancer genes were found to be under strong positive selection even in physiologically normal skin (Martincorena et al., 2015). Positively selected mutations were found in 18–32% of normal skin cells. The size of clonal expansions varied across genes, and gene size was not necessarily correlated with the potential of the somatic mutation to induce malignant transformation. Consistent with findings in tumours, the mutation rate also varied along the genome, with higher rates found in less frequently expressed genes and in repressed chromatin. These findings were inconsistent with the assumption that driver mutations occur infrequently in long-lived cell lineages and that those arising in cancer are too small to be detected clinically (Martincorena et al., 2015).

If a gene signature – a group of genes with a characteristic combined expression pattern – is associated with a prognosis, it is assumed to be likely to encode a biological signature driving carcinogenesis. However, this assumption has been questioned in view of findings that random changes in gene sets are associated with prognosis and that prognostic signatures in ER-negative breast cancer – associated with hypoxia and angiogenesis – are more similar to those in ovarian cancer than to those in ER-positive breast cancer, which are driven by proliferation pathways (Beck et al., 2013). Two distinct expression arrays of breast cancer cells, with almost no genes – and thus no protein changes – in common, can be equally useful for predicting clinical behaviour, and analyses with gene expression arrays may not provide a true understanding of cancer biology (Weinberg, 2014).

Sequencing of entire tumour genomes has yet to demonstrate definitively the number of somatic mutations required to create a human tumour. A few conceptual insights into cell and tissue behaviour have resulted from elaborate maps of interacting signalling components and computer models of signalling (Weinberg, 2010). The paradigm of somatic evolution and multistep tumorigenesis does not provide a logical reason why oncogenesis recapitulates ontogenesis (Huang et al., 2009).

Alterations in nutrient supply

Although dysregulated metabolism is one of the most common and recognizable features of cancer and is associated with other phenotypic indicators, the results of a recent literature review attempting to link cancer development and dysregulated metabolism suggested that there are major gaps in the understanding of exposure-related carcinogenesis and metabolic reprogramming, for example with respect to the specific causal and temporal relationships between exposures, dysregulated metabolism, and the development of cancer and the associated phenotypic hallmarks of cancer (Robey et al., 2015). This review did not consider lifestyle-related exposures and IARC Group 1 carcinogens.

It is difficult to identify associations that directly support a primary metabolic link between environmental exposures and cancer, for several reasons: metabolic control does not occur in a single step in a metabolic pathway; controlling factors differ between intact cells and in vitro cell-free systems; observed changes in individual pathway elements do not always lead to changes in metabolic flux; and cancer cell phenotypes are neither fixed nor cancer-specific. The review also noted the functional interdependence of dysregulated metabolism and other hallmarks of cancer, considering that, for example, proliferating cancer cells have shared regulatory factors associated with the fundamental anabolic and catabolic demands of the hallmark "sustaining proliferative signalling" (Robey et al., 2015).

Increased body mass index has been associated with increased risk of cancer: obesogens – chemicals that disrupt normal development and the balance of lipid metabolism – are able to cause permanent changes in metabolism that may render the subject more susceptible to cancer later in life (see Chapter 19, by Caldwell et al.). Inflammation is associated with metabolic changes and has been linked with several chronic diseases, including cancer. Extracellular pro-inflammatory metabolic signals are adenine nucleotides, succinate, oxidized nicotinamide adenine dinucleotide (NAD^+), and urate (McGettrick and O'Neill, 2013; Tannahill et al., 2013). The gut microbiome has an important role in carbohydrate absorption and metabolism in humans and plays a significant part in inflammatory responses as well (see Chapter 19, by Caldwell et al.).

The transgenerational character of metabolic disturbances and effects on cell signalling is demonstrated by studies of multigenerational undernutrition in rats (i.e. for 50 generations). The undernourished rats were predisposed to insulin resistance, had altered levels of several metabolic regulators (e.g. circulating insulin, homocysteine, endotoxin, leptin, adiponectin, vitamin B_{12}, and folate), and had a higher susceptibility to streptozotocin-induced diabetes compared with properly fed control rats. These changes were not reversed by feeding rats a normal diet in the two subsequent generations (Hardikar et al., 2015).

Studies on altered cell signalling have traditionally been performed on putative target cells of cancer, but the contribution of the gut microbiome (i.e. the microbiota living on and in humans) has only recently been investigated as a factor in cancer susceptibility (see Chapter 19, by Caldwell et al.). The microbiome plays a role in the control of nutrient supply (e.g. the gut microbiome is highly enriched in carbohydrate metabolism genes, compared with the human genome overall; Bultman, 2014), in metabolic pathways, and in host susceptibility to metabolic disease (Suez et al., 2014).

Cell proliferation as a component or cause of cancer

There are at least three scenarios related to cancer and mechanisms of cancer induction in which alterations in cellular replication and/or cell-cycle control have been described. The first invokes the predisposition of replicating cells with unrepaired DNA damage to develop into cancer cells. The second identifies sustained replication as a key event in various modes of action, and the third describes the ability of a transformed cell to escape normal cell-cycle control and to continue replication. The interpretation of mechanistic data for cell proliferation and cell death is dependent on the development of appropriate animal models (see Chapter 19, by Caldwell et al.), and although cell proliferation has been used in descriptions of hypothesized modes of action (Wood et al., 2015), it should be viewed in the context of the newer understandings of cancer mechanisms.

For DNA damage to lead to a mutation, DNA replication and cell division are typically required (see Chapter 12, by DeMarini). As noted in the United States Environmental Protection Agency guidance assessing the risk of cancer from early-life exposures (EPA, 2005), more frequent cell division during development can result in enhanced fixation of mutations because of the reduced time available for repair of DNA lesions, and clonal expansion of a mutated cell produces a larger population of mutant cells. For adult organisms, sustained cell proliferation has also been postulated to increase risk of cancer, based on the same rationale, and it has been proposed as a factor in increased cancer susceptibility. Sustained cell proliferation is a feature of several hypothesized modes of action for cancer development, for example the induction of kidney cancer via $alpha_{2u}$-globulin accumulation (EPA, 1991).

Although alterations in cellular replication or cell-cycle control are important features of carcinogenesis, cell proliferation in and of itself is not able to induce cancer. Several carcinogenic substances can cause cancer in humans after perinatal or prenatal exposure without the need for either continued exposure or sustained proliferation during exposure (see Chapter 19, by Caldwell et al.). It has been noted for some time that enhanced cell division does not always predict carcinogenesis (Melnick et al., 1993). After exposure to a carcinogen, the development of cancer in experimental animal models is influenced by the circumstances under which exposure occurs (e.g. sustained vs transient) and by the presence or absence of inflammatory mediators or DNA damage.

Liver cancer

The complex interactions between proliferation, mutation, and inflammatory cell signalling have been studied extensively for liver cancer. In humans, hepatocellular carcinoma (HCC) is markedly heterogeneous, both histomorphologically (Yeh et al., 2007) and genetically, with a wide diversity in gene expression patterns (Chen et al., 2002). Histopathological variability is also associated with geographical region: slow-growing, differentiated HCC nodules surrounded by a fibrous capsule are common in this type of cancer in Japanese patients, whereas a febrile form of HCC, characterized by leukocytosis, fever, and necrosis within a poorly differentiated tumour, is common in this cancer type among black people in South Africa (Feitelson et al., 2002).

HCC signature genes vary considerably and depend on etiological and accompanying pathological conditions, such as viral infection, cirrhosis, inflammation, and fibrosis. The study of tumour formation in the liver is also affected by continuous changes in the transcriptome that accompany hepatectomy and age (Colak et al., 2010). A comparison of

conserved genes between rats and humans (human orthologues) with respect to early expression profiles of HCC signature genes showed some conservation between species for components of the MAPK/ERK, phosphoinositide 3-kinase (P13K)/Akt, and transforming growth factor beta (TGF-β) pathways (Colak et al., 2010).

Development of liver cancer after exposure to carcinogens is more common in rodents, especially in the mouse, than in humans. There are obvious differences between rodents, non-human primates, and humans in background susceptibility to hepatocarcinogenesis and, as noted above, in the regulation of gene expression and cell signalling. With respect to the ability to respond to a mitogenic stimulus such as partial hepatectomy, the liver responds differently and much more slowly in non-human primates and in humans compared with rodents (Gaglio et al., 2002).

Global gene expression patterns in HCCs from seven different mouse models were compared with those in human HCCs from groups with poorer survival and better survival. Expression patterns in HCCs from *Myc-Tgfa* transgenic mice and in diethylnitrosamine-induced HCCs in mice were most similar to those in human HCCs from the group with poorer survival, whereas the patterns in HCCs from *Myc*, *E2f1*, and *Myc-E2f1* transgenic mice were most similar to those in human HCCs from the group with better survival (Lee et al., 2004).

Many factors, such as diet, hormones, oncogene activation, methylation, imprinting, and cell proliferation or apoptosis, are modulators of spontaneous and induced murine hepatocarcinogenesis. There is no simple paradigm to explain the differences in strain sensitivity, for example between C3H/HeJ and C57BL/6J mice, which show a difference of up to 40-fold in multiplicity of liver tumours (Hanigan et al., 1988; Maronpot, 2009).

The activation of oncogenic pathways appears to be more heterogeneous in human HCC than in other types of cancer (El-Serag and Rudolph, 2007). The high degree of heterogeneity in the ways in which cell signalling is disturbed before hepatocellular neoplasia may make induction of liver cancer a useful marker for changes that can lead to cancer elsewhere, depending on cellular context and target (Vogelstein and Kinzler, 2004).

Thus, pathway concordance between species may not always result in site concordance for expression of cancer. The analysis of liver tumour site concordance is complicated by the heterogeneity of disease in humans, as well as by rodent susceptibility.

For induction of cancer in the liver in rodents, the nature of cell proliferation also determines the risk of cancer (Caldwell et al., 2008). When both necrosis and inflammation are present, the resultant hepatocellular proliferation is fundamentally different from the transient proliferation caused by peroxisome proliferators or other primary mitogens. After treatment with a mutagenic agent, transient proliferation induced by primary mitogens has not been shown to lead to cancer induction, whereas partial hepatectomy or necrogenic treatments with carbon tetrachloride have (Ledda-Columbano et al., 1993; Gelderblom et al., 2001).

The mechanism by which necrosis may enable cancer development involves concurrent inflammatory cell signalling and is consistent with inflammation contributing to cancer development. After exposure of rodents to trichloroethylene, hepatocyte proliferation is confined to only a small population of cells without regenerative hyperplasia, sustained hepatocellular proliferation, and hepatocellular necrosis. Any transient DNA synthesis, peroxisome proliferation, or cytotoxicity is not correlated with trichloroethylene-induced liver carcinogenicity (EPA, 2011). Thus, induction of liver cancer by trichloroethylene is not a result of sustained cell proliferation.

Exposure to one of the most studied carcinogens, 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD), induces liver cancer in rodents, but short-term effects do not include induction of hepatocellular proliferation. Rather than simply inducing cell proliferation, TCDD is thought to cause cancer by altering the cellular ability to proliferate, migrate, undergo apoptosis, senesce, and terminally differentiate in a multistep process focused on the accumulation of mutations and heritable epigenetic changes (Safe, 2001; Marlowe and Puga, 2005; Ray and Swanson, 2009). In addition, the upregulation of drug-metabolizing enzymes by TCDD may enhance the formation of highly reactive intermediates during metabolic activation and/or transformation of several key hormones (e.g. enzyme induction as a source of reactive oxygen species formation, which is linked to decoupling of the cytochrome P450 catalytic cycle) and result in DNA damage and mutations (IARC, 2012c).

Although liver data provide an example of the role of inflammatory signals under some circumstances, inflammation in itself may not induce cancer without other concurrent

cofactors. For many of the agents discussed in Volume 100C of the *IARC Monographs* (IARC, 2012a), inflammation is a key characteristic of their effects (see Chapter 17, by Kane). One of the most recognized examples of how inflammation contributes to neoplastic development is the induction of mucosa-associated lymphoid tissue (MALT) lymphoma and gastric adenocarcinoma associated with exposure to *Helicobacter pylori.* The MALT proliferations of B-cell lymphoid follicles are the precursor of a low-grade lymphoma of B cells. A large proportion (98%) of patients with gastric MALT lymphoma are also infected with *H. pylori*; however, only a small percentage of *H. pylori*-positive individuals develop MALT lymphoma (Bassig et al., 2012). Little is known about the possible role of environmental cofactors in the predisposition to *H. pylori*-induced gastric lymphomagenesis. Other factors are certainly involved, including susceptibility (IARC, 2012b).

Inflammation

Certain types of inflammatory processes in skin, and possibly in other tissues, may serve a tumour suppressor function. Some clinical conditions show that inflammation is a critical component of tumour progression, for example reflux esophagitis before oesophageal cancer, or inflammatory bowel disease that precedes colorectal cancer. However, a condition such as psoriasis is known as a chronic cutaneous inflammatory disease that is seldom, if ever, accompanied by cancer. Similarly, despite extensive inflammation, activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and abundant proliferation of bile ducts in portal spaces, *Mdr2* knockout mice rarely develop tumours of the bile duct (Nickoloff et al., 2005).

The relationship between chronic inflammation and cancer is complex: inflammation may have roles in initial genetic mutations or epigenetic changes that not only drive cell transformation but also provide a microenvironment that enables progression and metastasis and prevents immune responses against the tumour. Chronic inflammation favours accumulation of DNA damage and chromosomal damage (see Chapter 12, by DeMarini, and Chapter 17, by Kane).

The hallmarks of cancer

In their updated paper, Hanahan and Weinberg (2011) noted that the most fundamental trait of cancer cells involves their ability to sustain chronic proliferation. As part of the "hallmarks" of cancer, alterations in cellular replication and/or cell-cycle control figure prominently in the discussions of cell proliferation, inflammation, and changes in cell signalling that are part of cancer cell physiology. The authors noted that the precise identities and sources of the proliferative signals in general remain poorly understood, but that mitogenic signalling in cancer cells is characterized and known in somewhat more detail. The mechanism by which necrosis enables cancer induction was also described in terms of the release of pro-inflammatory signals by necrotic cells and the influence of cytokines on proliferation and invasiveness of cancer cells. Thus, tumour-promoting inflammation was considered by Hanahan and Weinberg (2011) to be an enabling characteristic for acquisition of core hallmark capabilities.

Several key mechanistic characteristics of IARC Group 1 carcinogens induce traits of cancer cells described as the hallmarks of cancer, including effects on cell proliferation, cell death, and nutritional status (see Chapter 11, by Stewart). The description of the hallmarks attempts to bring together a fundamental understanding of how cancer cells manifest a distinct phenotype. More recently, a series of reviews in *Carcinogenesis* reported the findings from an international team of cancer biologists and toxicologists who participated in the Halifax Project (Harris, 2015). They reviewed the literature on each of the hallmarks of cancer to examine the carcinogenic potential of exposure to low doses and mixtures of chemicals. Relevant reviews for alterations in cell proliferation, cell death, or nutrient supply included the potential of chemical mixtures to enable sustained proliferative signalling (Engström et al., 2015), to confer resistance to cell death (Narayanan et al., 2015), and to induce metabolic reprogramming and dysregulated metabolism (Robey et al., 2015). A related review that encompasses many aspects of cell signalling reported on environmental immune disruptors, inflammation, and risk of cancer (Thompson et al., 2015). The overlap in the descriptions of pathway disruption and functions of cell signalling molecules between these papers is striking and is consistent with the discussion presented above with regard to the complex relationship and pleiotropic roles of cell signalling molecules involved in cell proliferation and apoptosis.

The paradigms of cytotoxicity, cell proliferation, and initiation–promotion as mechanisms of carcinogenesis have been superseded by a more nuanced understanding of the

process of carcinogenesis (Hanahan and Weinberg, 2011). As stated above, cell proliferation, inflammation, or cytotoxicity alone do not lead to cancer, and they are interrelated through changes in cell signalling. The hallmarks of cancer describe well the characteristics that are manifested after the development of cancer. However, by the time of diagnosis, tumour cells already carry large numbers of mutations and are also very heterogeneous in gene product profiles; the multiple cell divisions and the consequent damage processing obscure the initial lesion, rendering it difficult, if not impossible, to make a distinction between causal and consequential events in carcinogenesis.

Conclusions

Among the 10 mechanistic characteristics more commonly observed for IARC Group 1 carcinogens is a composite that includes information on alteration of cell proliferation, cell death, and nutrient supply. This chapter examines many of the challenges associated with the use of this type of information to determine mechanistic and tumour site concordance between humans and experimental animals, and discusses how this mechanistic characteristic shows interdependence with others, such as genotoxicity and inflammation. Many of the indicators of changes in cell proliferation or cell death are non-specific for the induction of cancer, and although they result primarily from effects on the MAPK/ERK cell signalling pathway, they are influenced by a large array of cell signalling molecules with pleiotropic effects.

The biological basis for how cell signalling and cell-cycle control differ between species, organs, and tumour cells, as well as the variability in mutation targets, are also discussed here. Determining the causes of cancer through examination of gene expression profiles in tumours is difficult, especially in terms of increased and sustained cell proliferation, which is a characteristic of cancer itself. Alteration in nutrient supply is a common and recognizable feature of cancer, and is also not independent of activities associated with increased cell proliferation or the hallmarks of cancer.

Some of the information collected in Volume 100 of the *IARC Monographs* was presented in the context of the older hypotheses for mechanisms of cancer induction. The understanding of cancer mechanisms and the descriptive data associated with them continues to evolve (see Chapter 11, by Stewart). The discussion of mechanistic data for ionizing radiation in Volume 100D of the *IARC Monographs* (IARC, 2012d) and Chapter 12, by DeMarini, provide more current discussions about the understanding of cancer and the key mechanistic characteristics of IARC Group 1 carcinogens. With the present state of knowledge, carcinogenesis cannot be confidently attributed to an underlying purely genetic or purely epigenetic process. Mechanistically, it is probably a mixture of the two.

Epigenetic alterations may precede DNA sequence mutations, with subsequent mutations occurring not in a random fashion but in response to specific types of epigenetic changes induced by the environment (Karpinets and Foy, 2005). This selection for enhanced growth has been suggested to explain both the delayed cancer induction after exposure to toxicants and the bystander effect of radiation on tumour development. With regard to cell signalling, spontaneous or environmentally induced epigenetic alterations are increasingly recognized as early molecular events in cancer formation, and these alterations may potentially be more adverse than nucleotide mutations, because their effects on regional chromatin structure can spread out, thereby affecting multiple genetic loci (Weidman et al., 2007).

The key characteristics of the IARC Group 1 carcinogens have some overlap with the hallmarks of cancer and perhaps can provide insight into the "environment" that creates the neoplastic cell. The elucidation and understanding of susceptibility factors may help determine what parts of that environment are already present in individuals, species, or target tissues where cancer develops as a result of exposure to environmental carcinogenic agents. This may also help in evaluating mechanistic and tumour site concordance between species. However, the mechanistic data provided on agents identified as *carcinogenic to humans* need to be examined in the context of more recent information on carcinogenesis. Cancer is a heterogeneous disease, even within the same target site. Because, among other considerations, some epigenetic events may have a mutational basis, the dichotomy once identified between genotoxic and non-genotoxic carcinogens should be reconsidered.

Enhanced cell proliferation and reduced cell death are key mechanistic characteristics of IARC Group 1 carcinogens, are a hallmark of cancer, and are necessary for DNA damage to be processed into a mutation.

However, such changes alone are not sufficient to induce cancer. A key mechanistic question emerges as to what events or cellular environment may precede or cause such changes and may stimulate the formation and selection of DNA sequence mutations and epigenetic changes that induce a cell and its descendants to acquire the hallmarks of cancer, including increased cell proliferation and evasion of apoptosis.

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