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Review

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REVIEW

Causes of genome instability: the effect of low dose chemical exposures in modern society

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Abstract

Genome instability is a prerequisite for the development of cancer. It occurs when genome maintenance systems fail to safeguard the genome's integrity, whether as a consequence of inherited defects or induced via exposure to environmental agents (chemicals, biological agents and radiation). Thus, genome instability can be defined as an enhanced tendency for the genome to acquire mutations; ranging from changes to the nucleotide sequence to chromosomal gain, rearrangements or loss. This review raises the hypothesis that in addition to known human carcinogens, exposure to low dose of other chemicals present in our modern society could contribute to carcinogenesis by indirectly affecting genome stability. The selected chemicals with their mechanisms of action proposed to indirectly contribute to genome instability are: heavy metals (DNA repair, epigenetic modification, DNA damage signaling, telomere length), acrylamide (DNA repair, chromosome segregation), bisphenol A (epigenetic modification, DNA damage signaling, mitochondrial function, chromosome segregation), benomyl (chromosome segregation), quinones (epigenetic modification) and nano-sized particles (epigenetic pathways, mitochondrial function, chromosome segregation, telomere length). The purpose of this review is to describe the crucial aspects of genome instability, to outline the ways in which environmental chemicals can affect this cancer hallmark and to identify candidate chemicals for further study. The overall aim is to make scientists aware of the increasing need to unravel the underlying mechanisms via which chemicals at low doses can induce genome instability and thus promote carcinogenesis.

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Abbreviations

5hmC	5-hydroxymethylcytosine
5mC	5-methylcytosine
BC	black carbon
BER	base excision repair
BPA	bisphenol A
CNV	copy number variation
CYP	cytochrome P450
DNMT	DNA methyltransferase
HDAC	histone deacetylase
IARC	International Agency for Research on Cancer
miRNA	micro RNA
MMR	mismatch repair
	mRNA; messenger RNA
MSI	microsatellite instability
mtDNA	mitochondrial DNA
mtDNAcn	mtDNA copy number
NER	nucleotide excision repair
NF-κB	nuclear factor-kappaB
NP	nanoparticle
PAH	polycyclic aromatic hydrocarbons
ROS	reactive oxygen species
SNP	single-nucleotide polymorphism
TET	ten-eleven translocation
WC	tungsten carbide

Introduction

The understanding of cancer as a genetic disease has dramatically evolved during the last few decades with the knowledge that cancer cells acquire their characteristics at different times during cancer development, in diverse microenvironments, via various mechanisms (1,2). It is increasingly clear that the phenotypic variations underlying cancer result from multiple interactions among numerous environmental and genetic factors that occur over long periods of time (3). Molecular epidemiological studies (4–14) have shown interesting associations between certain single environmental exposures and early effects related to carcinogenesis. However, we know surprisingly little about the cancer risks that might be attributable to the combined effects of the many chemicals that we encounter in our everyday lives at low doses. Consequently, the non-governmental organization 'Getting to Know Cancer' (gettingtoknowcancer.org) invited scientists to prepare the current review, requesting them to examine the possibility that environmental chemicals that are not considered as class I carcinogens [according to the International Agency for Research on Cancer (IARC)] could still indirectly contribute to genome instability and carcinogenesis.

Genome instability is defined as an increased tendency of the genome to acquire mutations (15). It occurs when various processes involved in maintaining and replicating the genome are dysfunctional or when there is increasing exposure to carcinogens. Genome instability is an enabling characteristic that is causally associated with the acquisition of cancer hallmarks. The mechanisms leading to genome instability and enhanced mutation frequency include inherited or acquired defects in DNA repair, DNA replication, cell cycle control or chromosome segregation. Genome instability is indicated (and measured) by an elevated frequency of simple or complex mutations to the genome, namely changes in the nucleotide sequence (base substitutions, base loss, nucleotide deletion, insertion or amplification), DNA breaks, chromosomal translocations, inversions and deletions and abnormal numbers of chromosomes (aneuploidy). Altogether, this instability plays an essential role in carcinogenesis (16–18) (Figure 1). Exogenously induced DNA damage, caused by a variety of environmental chemicals, nanoparticles (NPs), radiation or biological agents, can cause loss of genome integrity, and also endogenously induced (metabolically caused) DNA damage is significant. It is estimated that DNA lesions of endogenous origin occur thousands of times daily in the genome of every human cell (19). In general, various DNA repair pathways prevent the persistence of such DNA lesions, maintaining genome integrity. Gene mutations, defined as irreversible

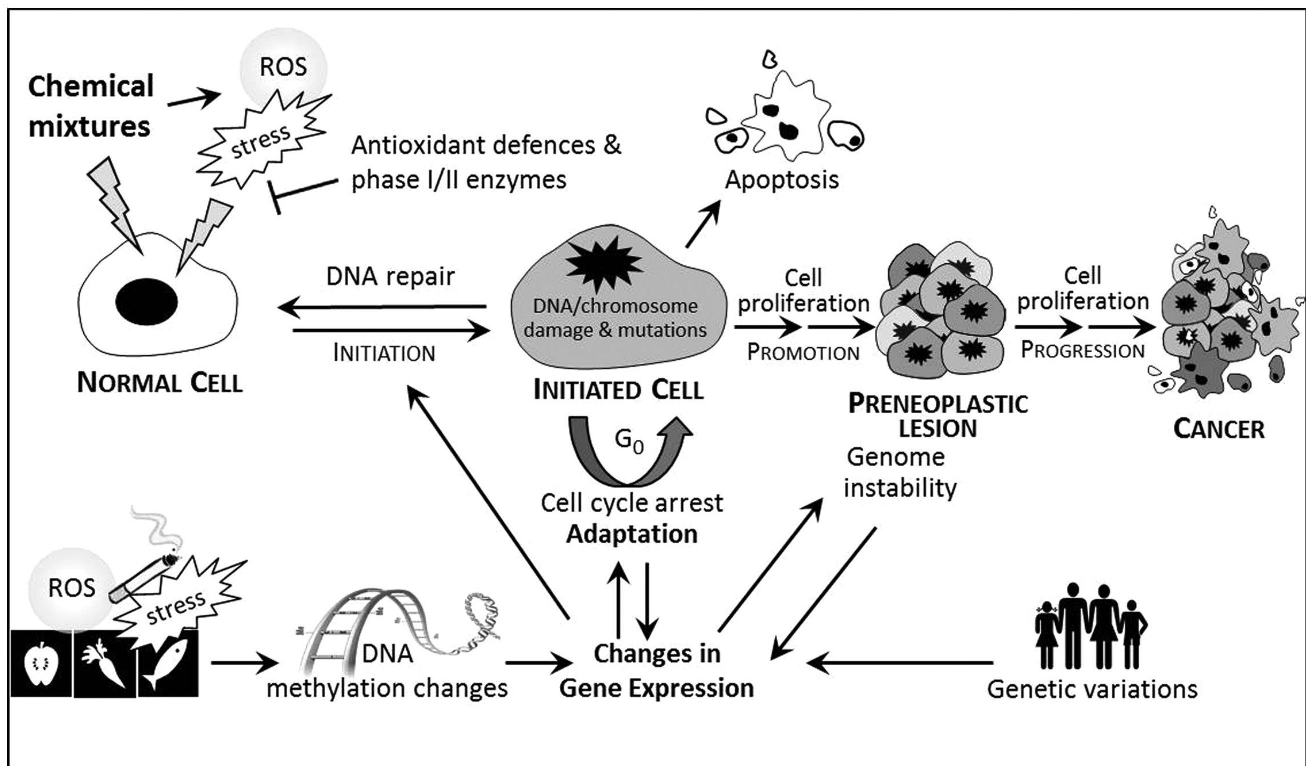

Figure 1. Cellular mechanisms linking exposures to chemical mixtures with cancer. Effects of chemical mixtures may be modulated by various pathways and mechanisms, including enzymic and non-enzymic antioxidants, phase I and II metabolizing enzymes, cell cycle arrest, DNA repair, apoptosis, and by epigenetic mechanisms (such as DNA methylation) that can regulate gene expression. Defense mechanisms limit the accumulation of DNA damage and reduce the risk of genome instability (mutations, chromosomal anomalies, telomere shortening etc.) and the progression to cancer. It should be noted, however, that phase I metabolism can result in the transient presence of activated carcinogens.

changes in the nucleotide sequence, arise much more rarely. They are a result of misrepair or erroneous replication of the nucleotide sequence containing the lesion. If mutations occur in certain genes that play essential roles in the finely tuned control of DNA integrity, altered expression (e.g. of oncogenes or tumor suppressor genes) may take place, with (among other things) genome instability as a consequence. Moreover, changes to the epigenome may also lead to genome instability in an indirect way. For example, epigenetic alterations can (i) affect DNA repair efficiency and fidelity by changing the expression of DNA repair genes (20–24); (ii) induce chromatin disruption (25,26) and (iii) deregulate enzymes involved in antioxidant defenses or phase I/phase II xenobiotic metabolism (27–29).

Here, we describe different aspects and causes of genome instability, referring to the various biological targets that maintain genome integrity. Furthermore, we discuss a selection of chemicals that can deregulate the genome maintenance systems, adding to the burden of genome instability and thus increasing cancer incidence.

Causes of genome instability and levels of dysfunction

Chemicals, radiations and biological agents (e.g. viruses, bacteria and parasites) can have direct effects on genome integrity, causing specific DNA lesions [e.g. 8-oxo-7,8-dihydroguanine (8-oxoG), cyclobutane pyrimidine dimers, bulky adducts], inducing gene mutations and chromosome aberrations. Environmental exposure can also cause genome instability via indirect mechanisms; these could involve single-nucleotide polymorphisms (e.g. SNPs in chemical metabolism, DNA repair, cell cycle), effects on DNA repair enzyme activity, changes in nuclear and mitochondrial DNA copy number (mtDNAcn) or microsatellite instability (MSI), changes in epigenetic regulation of gene expression (e.g. differential promoter region methylation) or altered levels of specific proteins (30). Such indirect effects form the basis of our criteria for selecting the chemicals described in Chemical disruptors and their effect on biological target sites, but first we give an overview of the process of carcinogenesis insofar as it involves changes to or modifications of the genome.

Direct effects on genome integrity

When DNA damage is not repaired by the time a cell comes to replicates its DNA, it can give rise to gene mutations (base-pair alterations, deletions and insertions), and in turn chromosome aberrations (when, e.g., a double-strand break or large deletion is present at mitosis). These can all be regarded as direct effects on genome integrity.

DNA damage, gene mutations, oncogene activation and tumor suppressor inactivation

For decades, DNA damage has been considered as the most likely event to 'kick-start' the multistep carcinogenic process (31). Of all the chemicals classified as carcinogenic to humans by the IARC, a great proportion have been shown to exert their biological effects through binding of their DNA-reactive metabolites forming covalent DNA adducts or through base modifications (e.g. oxidation, alkylation). DNA adduct analyses reflect tissue-specific rates of adduct formation and removal, which depend on carcinogen uptake, metabolic activation, DNA repair,

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adduct instability and tissue turnover and are thus useful markers of exposure to DNA-reactive chemicals and, to a certain extent, of cancer risk.

The various forms of DNA damage caused by radiation and exogenous or endogenous chemicals include single-strand breaks, double-strand breaks, apurinic/apyrimidinic sites, oxidation or alkylation of bases, bulky adducts (large chemical groups covalently bound to DNA bases), intra-strand cross-links (such as dimerization of adjacent pyrimidines), inter-strand cross-links (covalent links between the two strands of the double helix) and covalent bonds between DNA and protein. In addition, mismatches can occur through slipped alignment of microsatellite repeats and faulty replication, especially if an apurinic/apyrimidinic site or a base with altered base-pairing properties (caused by oxidation or alkylation, or a bulky adduct) is present when the DNA is replicated (32).

The development of cancers can be mediated through genetic (or epigenetic) alterations in oncogenes and tumor suppressor genes that regulate processes such as cell proliferation, cell death, cell differentiation and genome stability. Most oncogenes result from proto-oncogenes that are normal functional genes. When a proto-oncogene mutates at its critical DNA sequences or increases its expression, it becomes an oncogene, leading to cancer. This process is called oncogene activation. Examples of proto-oncogenes include *RAS* regulating growth signaling, and *MYC*, a gene coding for a regulatory transcription factor (33). Tumor suppressor genes, on the other hand, are normal genes that inhibit cell growth, stop cell division and promote DNA repair or apoptosis when a cell's DNA is damaged. When mutations occur in the tumor suppressor genes, cells can grow out of control, which can lead to cancer. Examples of tumor suppressor genes include DNA repair genes, *BRCA1* and *BRCA2*, and *TP53*, which is mutated in most human cancers (34).

Oncogenes are generally activated through mutations, gene amplification or chromosome rearrangements; only one of the two copies of a proto-oncogene needs to be activated since mutations are dominant. These three mechanisms result in either an alteration of proto-oncogene structure or an increase in proto-oncogene expression (35). Structural alterations in the encoded proteins often lead to the uncontrolled, continuous activity of the mutated protein. For example, *RAS* mutations induce uncontrolled cell division (36,37). Gene amplification occurs through replication of a DNA region containing an oncogene and can result in several hundred copies of the gene. Thus, the expression level of the oncogene increases, conferring a selective advantage for cell growth. About 20–30% of breast and ovarian cancers show c-*MYC* amplification (38). Chromosomal rearrangements consist of translocations and inversions. Chromosomal rearrangements can move a proto-oncogene to a site close to an immunoglobulin or T-cell receptor gene. Transcription of the proto-oncogene is then under control of regulatory elements from the immunoglobulin or T-cell receptor locus, leading to deregulation of the proto-oncogene expression. A well-known example is the *BCR–ABL* fusion gene, the Philadelphia chromosome, present in most cases of chronic myelogenous leukemia (39).

Since mutations in oncogenes generally affect other hallmarks of cancer (e.g. cell growth as described above), there are doubts as to whether oncogenes do induce genome instability. However, activation of oncogenes, and as a result growth signaling pathways, has been reported to induce loss of heterozygosity and genome instability in various *in vitro* and *in vivo* models, via a mechanism involving DNA replication stress [for review (40)].

In contrast with oncogene activation, tumor suppressor genes cause cancer when they are inactivated. Since there are normally two copies of a tumor suppressor gene per cell, loss-of-function mutations that occur in one copy of a tumor suppressor gene cannot inactivate the whole functions of the tumor suppressor gene because the other copy of the gene continues to produce functional proteins. Thus, mutations in tumor suppressor genes are considered recessive. Recent studies show that loss of a chromosomal segment harboring a haploinsufficient tumor suppressor gene can lead to reduced gene expression and is sufficient to contribute to tumorigenesis (41). Oncogene activation and tumor suppressor inactivation tend to enhance tumorigenesis cooperatively (42).

In addition to the genetic mechanisms, activation of oncogenes and inactivation of tumor suppressor genes can be instigated through epigenetic mechanisms as described below (Epigenetic regulation).

Structural and numerical chromosomal changes

Alterations to the chromosomes can be structural, including translocations, deletions, insertions, inversions, breaks, sister chromatid exchange, micronuclei, chromothripsis and changes in telomere length. Numerical changes in chromosome copy numbers can also occur as is the case in aneuploidy and polyploidy (43). Both structural and numerical aberrations are frequently observed in cancers. For example, the frequency of sister chromatid exchange (the process whereby, during DNA replication, two sister chromatids break and rejoin with one another, physically exchanging regions of the parental strands in the duplicated chromosomes) is almost 10-fold higher in Bloom syndrome patients with a predisposition to develop cancer, compared with healthy individuals (44).

Aneuploidy refers to an aberrant chromosome number that deviates from a multiple of the haploid set. Constitutional aneuploidy is present in ~0.3% of newborns. Mosaic aneuploidy (i.e. appearing in only a fraction of an individual's cells) is common in neurons of normal mice and humans and in mammalian hepatocytes. In these liver cells, an age-dependent increase in polyploidization occurs as aneuploid cells divide [for review (45)]. Several mechanisms are involved in mal-segregation of chromatids among both daughter cells during mitosis: cohesion defects of sister chromatids, merotelic attachment of chromosomes, hyperstabilization of kinetochore–microtubule interactions, tubulin depolymerization, dysfunctional telomeres, defects in mitotic checkpoints and induction of unstable tetraploid intermediates by mitotic slippage, cytokinesis failure or viral-induced cell fusion [for review (46–49)]. Mutagens that are able to induce aneuploidy are called aneugens. Aneuploidy can induce a 'mutator phenotype' that increases DNA damage and genome instability.

The major consequences of aneuploidy [reviewed in [45,50]] are (i) reduction of cellular fitness and the development of the organism by repression of cell proliferation; (ii) modification of the proteome leading to increased energy burden, alteration of metabolic capacities, increased drug sensitivity and increased likelihood of senescence; (iii) trading a reduction in proliferation rate for an increased ability of aneuploid cells to adapt and evolve, called the aneuploidy paradox and (iv) promotion of genome instability by creating imbalance in the levels of proteins required for DNA replication, repair and mitosis or by induction of chromosome breaks in the lagging chromosome trapped in the cleavage furrow during cytokinesis. In addition, lagging of chromosome(s) during the metaphase/anaphase transition may produce a micronucleus [for review (49)]. Micronuclei can

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undergo rearrangements by two possible mechanisms [reviewed in (50)]: (i) chromosome shattering (chromothripsis) results from mitotic entry—before completion of DNA replication within the micronucleus—and a failure to disassemble the micronuclear envelope encapsulating the chromosomal fragments for random reassembly in the subsequent interphase or (ii) locally defective DNA replication initiates serial, microhomology-mediated template switching (called chromoanagenesis) that produces local rearrangements with altered gene copy numbers.

Boveri (51) was the first to postulate that misdistribution of chromosomes might be a cause of tumor development and birth defects. Whether aneuploidy is a cause or a consequence of carcinogenesis has been a matter of debate for many years, but today it can be stated that both are true. Constitutional aneuploidies show inhibition of cell proliferation with the potential for tumor suppression but, on the other hand, are associated with alterations in the risk of specific cancers and therefore support a causative role of aneuploidy in cancer [reviewed in (52)]. Aneuploidy is a remarkably common feature of human cancer, present in ~90% of solid tumors and >50% of hematopoietic cancers. Although the degree and spectrum of aneuploidy vary considerably among tumor types, many show recurrent whole-chromosome aneuploidies (53). The role of aneuploidy in tumorigenesis has been analyzed in several studies [reviewed in (45,54,55)]. Although aneuploidy correlates with transformation, empirical tests of the hypothesis that aneuploidy drives tumorigenesis have been hampered by the difficulty of generating aneuploidy without causing other cellular defects, particularly DNA damage. In the light of our present knowledge, it seems likely that aneuploidy can contribute to carcinogenesis, without being a sufficient condition for malignant tumoral transformation.

Telomere shortening has been associated with aging, and this process can be accelerated by increased oxidative stress and episodes of inflammation (56). Evidence is rapidly growing that telomere length may be affected by environmental chemicals that have frequently been associated with chronic diseases (57). Moreover, previous findings (58) showed a potential unifying mechanism connecting the nucleus and mitochondria in cellular aging. In that work, progressive nuclear telomere shortening—mediated by the activation of a p53-dependent pathway—was found to determine a reduction of mitochondrial function and mtDNAcn (58).

Accumulating evidence has revealed that telomere dysfunction makes a significant contribution to genome instability in human cancer (59,60). In general, continued proliferation of human cells requires maintenance of telomere length, usually accomplished by telomerase. Ninety percent of human tumors are telomerase-positive (61). In normal cells, extended proliferation leads to telomere erosion and eventual loss of telomere function, so-called telomere crisis. Most epithelial cells, lacking active telomerase, undergo telomere erosion, become genomically unstable and eventually die.

Caretakers of genome integrity

The integrity of the genome is crucial for cancer avoidance and for the propagation of genetic information to subsequent generations. The 'caretakers' of genome integrity consist of (i) phase I (hydrolysis, reduction, oxidation) and phase II (glucuronidation, sulfation, acetylation, methylation, conjugation with amino acids or with glutathione) metabolizing enzymes that can process, usually inactivate or intercept the mutagenic agents and thus prevent DNA damage; (ii) DNA repair pathways dealing with different classes of lesions and (iii) signaling molecules that detect DNA damage and activate defense checkpoints (Figure 1).

Antioxidant defense systems as a first line of defense

The main antioxidant defenses are: glutathione [a sulfhydryl-rich compound that binds reactive oxygen species (ROS) and free radical intermediates], enzymes linked to glutathione (such as glutathione peroxidase), superoxide dismutase (breaking down superoxide into H_2O_2 and oxygen) and catalase (breaking down H_2O_2). These 'caretakers' are generally effective, but if ROS are present in excess, they can overwhelm the cellular defenses and lead to 'oxidative stress' (62,63). Phase I and phase II metabolizing enzymes play a crucial role in eliminating toxic chemicals from the body. Phase I enzymes (P450 cytochromes, also known as mixed function oxidases) typically oxidize xenobiotics to a reactive form, which—with the help of phase II enzymes such as glutathione S-transferase—is then conjugated to a carrier molecule such as glutathione and eliminated (64). However, as a side effect, the phase I enzymes are also responsible for activating certain non-carcinogenic chemicals to form DNA-reactive molecules that can potentially induce mutations. Polymorphisms in genes coding for these enzymes are very common, and so individuals can vary significantly in their resistance to the effects of genotoxic chemicals.

DNA repair

DNA repair is the second line of defense in the prevention of chemical carcinogenesis, acting on DNA damage that occurs in spite of other cellular defenses. The amount of DNA damage induced by an agent is usually linear in relation to the dose (65,66), but the extent to which DNA damage leads to mutations differs as a function of the activity and fidelity of DNA repair mechanisms. Single-strand breaks are repaired by a process that involves mainly end-processing and DNA ligation; consequently, in most cell types, they have a short half-life (a few minutes) and are regarded as relatively harmless (67). Small base changes (oxidation or alkylation) are repaired by base excision repair (BER), in which more or less specific glycosylases cut out the altered base, leaving an apurinic/apyrimidinic site that is incised and converted to a small DNA gap, followed by DNA polymerization and ligation (68). A half-life of a few hours is typical for these small base changes. Bulky adducts, pyrimidine dimers and inter-strand cross-links are dealt with by nucleotide excision repair (NER), which involves dual incisions on either side of the lesion, leaving a gap of around 28 nucleotides, filled by a DNA polymerase and ligated—again, with a half-life of a few hours. Double-strand breaks can be repaired either by homologous recombination (occurring during S phase, using the sister chromatid for sequence matching) or by non-homologous end-joining, which carries a risk of loss of a DNA sequence from the broken ends and is therefore potentially mutagenic (69). Mutations in genes encoding mismatch repair enzymes such as MSH2 and MLH1 are known to be involved in colorectal cancer (70,71).

Despite active DNA repair enzymes and delays imposed by cell cycle checkpoints, DNA damage may still be present when the cell replicates its DNA. Mechanisms have evolved that deal with such situations by bypassing the damaged site, allowing synthesis of a nascent DNA strand opposite the blocking lesion (72), although at the price of a high risk of errors, leading to mutation.

Interactions between environmental, nutritional and genetic factors can influence, for good or ill, the efficiency or fidelity of the various DNA repair processes. Polymorphisms in genes that code for proteins involved in BER or NER have been shown to influence enzyme activity (73) and have been linked to cancer risk (see Single-nucleotide polymorphisms). They probably

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account for a fraction of the inter-individual variation seen in repair capacity, and other factors such as induction or inhibition by dietary or environmental exposures need to be invoked to explain the bulk of the variation, which amounts to a range of several fold (74,75).

The importance of DNA repair for maintaining genome integrity and preventing the development of a neoplastic phenotype is underscored by the fact that defective DNA repair is linked to increased susceptibility of cells to toxic, mutagenic and carcinogenic effects of environmental exposures (76). This is, for example, illustrated by autosomal recessive disorders such as xeroderma pigmentosum photosensitive patients. Mutations in XP genes lead to defective NER and increased skin and thyroid cancer rates (76,77). Xeroderma pigmentosum is rare, but more frequent are patients with mutations in mismatch repair genes, which are responsible for hereditary and sporadic colon cancer (70,78).

Certain forms of DNA damage (such as those caused by ultraviolet radiation) occur only episodically, and the relevant DNA repair mechanisms appear to be inducible (directly or indirectly) by the corresponding types of damage (79–81). However, not all DNA repair mechanisms are inducible: some forms of DNA damage (notably oxidation) are omnipresent in mammalian or human cells (19), and the corresponding repair mechanisms are consequently permanently active.

The importance of removing DNA oxidation products is reflected in the redundancy of DNA repair enzymes for these lesions. Because of this redundancy, defining the role of ROS in cancer development and of the repair enzymes in preventing it is challenging. In any case, environmentally induced interference in the repair of such damage may contribute to cancer development. One example of a connection between ROS induction and cancer development is the increased incidence of cancers of the lung, skin, bladder and liver in humans who have been exposed to the ROS-inducing agent arsenic (82). In addition, reactive nitrogen species such as exogenous nitric oxide and peroxynitrite have been shown to inhibit the BER enzyme 8-oxoguanine DNA glycosylase (83) as well as DNA ligase (84). Moreover, it has been reported that NER can be inhibited by oxidative stress (85,86) and lipid peroxidation products (e.g. 4-hydroxynonenal, malondialdehyde) (87,88), most likely by direct oxidative attack and inactivation of NER proteins.

Although it is generally assumed that a high intrinsic ability to repair DNA will be protective against cancer, it is also possible that a measured high repair capacity reflects induction by exposure to DNA-damaging agents in the environment—in which case the precise link between repair capacity and cancer risk is less well understood.

DNA damage signaling

ROS, in addition to causing direct DNA damage, can also activate signal transduction pathways and induce transcription factors [e.g. *nuclear factor (erythroid-derived 2)-like 2 (Nrf2)*, nuclear factor-kappaB *(NF-κB), p53*] involved in regulation of various genes including DNA repair genes.

Activation of the p53 tumor suppressor will only occur after exposure to a certain dose of an exogenous agent, but not after exposure to very low doses. After activation of the p53 tumor suppressor, the 'global genomic repair' arm of NER is induced (80). However, there is no consensus on the amount of damage necessary for the activation of p53 (89–91).

Nrf2 is a master regulator of a battery of defensive and detoxification genes, through a series of signaling events, removing damaged proteins and promoting the overall survival of the cell. The precise role of Nrf2 in cancer remains controversial. Nrf2 acts as an anticancer protein that has been found to be both upregulated by tumor suppressor proteins and targeted for degradation by oncoproteins (92). Nrf2 also acts as a tumor promoter, as was concluded on the basis of various studies reviewed in Shelton *et al.* (92). Aberrant overexpression of Nrf2 in many cancers (produced by mutations), along with its ability to induce resistance to chemotherapeutic drugs, supports the notion that Nrf2 also acts as a tumor protein (93). Many chemopreventive agents are known to activate Nrf2 while repressing NF-κB activity. The publication of the Nrf2 interactome and regulatome highlights a vast array of potential proteins involved in the regulation of this pathway including NF-κB (94). The interplay between these two cellular defense pathways suggests that there is a coordinated protective response to cellular insults, but the exact mechanisms remain to be determined.

As the transcription factor NF-κB is inducible and transiently active downstream of a physiologically important and stress-induced pathway, it is not surprising that its deregulation is associated with promoting cancer. In addition, many viruses achieve their oncogenic effects via the NF-κB signaling cascade (95). A great variety of stimuli are able to induce the NF-κB pathway, among them pro-inflammatory cytokines tumor necrosis factor α and interleukin-1β, and also DNA-damaging agents such as ultraviolet light and some anticancer drugs. On the other hand, the NF-κB pathway drives the expression of over 100 genes including pro-inflammatory, anti-apoptotic and other important genes related with cancer development (vascular endothelial growth factor in angiogenesis, matrix metalloproteinase in metastasis and even important metabolism genes such as *γ-glutamyl-cysteine ligase (γ-GCL)*, *multidrug resistance 1 (MDR1)* gene and *superoxide dismutase (SOD2)*) (95,96). NF-κB functions downstream of the tumor suppressor p53, suggesting that NF-κB, under certain conditions, also functions as a tumor suppressor.

In chronically inflamed tissues, the neutrophils and macrophages secrete large amounts of ROS/reactive nitrogen species, recruiting more activated immune cells, in a 'vicious cycle', exacerbating the oxidation of intracellular proteins, lipids and nucleic acids and resulting in possible genetic changes and/or epigenetic alterations, which can lead to gene deregulation and initiation of carcinogenesis (97,98).

Other modes of cell signaling and their aberrant functioning in tumorigenesis will be discussed in more detail in several other articles in this special issue.

Modifiers of the mechanisms maintaining genome integrity

Inter-individual differences in response to environmental agents can be due to genetic and phenotypic variations in the metabolism of carcinogens and DNA repair efficiency. In the past decade, much interest has been directed to the role of epigenetic mechanisms in the carcinogenic process. Epigenetic modifications (e.g. aberrant DNA methylation patterns) do not require mutation of the genome to silence the expression of cell cycle regulators or to reduce the efficiency of DNA repair processes, which may also initiate carcinogenesis. Epigenetic alterations are seen as indicators of susceptibility to environmental exposures and are increasingly being studied in association with disease outcomes (99).

Single-nucleotide polymorphisms

The human genome project revealed that ~99.9% of human DNA sequences are the same between individuals. Only the remaining small part of approximately 0.1% of the human genome can

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vary in sequence between individuals (100). About 90% of these variations in the human genome consist of SNPs. DNA sequence variations are considered as SNPs when a single nucleotide (A, T, C or G) in the genome sequence is altered and the frequency of the variant allele in the population is at least 1%. SNPs that fall within coding sequences of genes can give rise to a protein that has an amino acid substitution or is truncated, leading to changes in activity, localization or stability (101,102). SNPs that do not occur in protein-coding regions may still have biological consequences. For instance, polymorphisms in promoter regions may affect the transcriptional regulation and level of expression of a protein, whereas SNPs in untranslated regions or near intron–exon junctions may disturb the messenger RNA (mRNA) structure and translational process or cause alterations in mRNA splicing, respectively (103). These genetic variations can affect an individual's phenotype and may predispose people to diseases such as obesity, cancer and cardiovascular disease. However, the accuracy with which these SNPs predict the phenotype is unknown in most cases (104,105). Moreover, the genetic profile of a person may affect their susceptibility toward exposure to chemical carcinogens. The volume of data available on genetic variations has increased considerably with the recent development of high-density SNP arrays. However, a number of issues are uncertain; for example, whether the alleles of several SNPs interact with one another, leading to a higher susceptibility to disease, or whether the individual risks associated with certain SNPs and environmental factors lead to more than additive risk profiles. Additionally, there is little information available on how the cancer risk due to these SNPs compares with the risk from environmental and occupational exposure to carcinogens.

Intrinsic variation as well as environmentally induced regulation of genes involved in xenobiotic metabolism can modulate the extent of exposure to reactive chemicals and damage at the target site and so can affect the risk of cancer. Exogenous substances are excreted following metabolic transformation by enzymes catalyzing oxidative activation (phase I) and inactivation by conjugation (phase II). Several of the 200 different known *cytochrome P450 (CYP)* genes code for proteins involved in phase I biotransformation processes. For each of the phase II enzymes, such as glutathione S-transferase, uridine 5′-diphospho-glucuronosyltransferase, *N*-acetyltransferase and sulphotransferases, several different isozyme forms are known. By 1998, ~10 polymorphisms were known to have an influence on the carcinogenic properties of exogenous substances (106). In addition to this list, Nebert (107) identified SNPs in *CYP1A2* and in the aryl hydrocarbon receptor that are linked with carcinogenesis. Hussain *et al.* (108) mention that the frequencies of alleles of biotransformation genes associated with increased risk vary between 2 and 50 per 100 births (depending on the type of detoxification gene). The increase in cancer risk associated with a risk allele amounts to a factor between 2 and 10. A combination of phase I and phase II polymorphisms can lead to a higher risk than a single polymorphism. These putative genetic biomarkers of susceptibility have severe limitations when we consider that a multiplicity of metabolic pathways is operating concurrently at different rates; it is therefore hard to predict the ultimate carcinogen-modulating effect by assessing just one or a few metabolic pathways (109).

Variation in expression and/or structure of enzymes involved in DNA repair pathways may influence the persistence of DNA damage. The majority of genes encoding for DNA repair proteins are polymorphic (110). Common SNPs in DNA repair genes have been associated with cancer risk (111–113). Several SNPs in NER and BER genes were shown to modulate the levels of chromosomal damage, measured as micronucleus frequencies (114–116). Moreover, the same SNPs that appear to influence cancer risk have been associated with changes in DNA repair activity (117,118). In general, however, studies regarding associations between SNPs and cancer risk or DNA repair are inconclusive (119). The diverse findings may be attributed to differences in study size and statistical power, as larger studies having greater power can detect smaller effects. It seems that either the ability of the candidate gene approach to identify genetic risk or the importance of DNA repair pathways may have been overestimated, considering that the risk conferred by single variants has been shown to be small in genome-wide association studies (120). However, environmental exposure is only now beginning to be considered as a covariate in genome-wide association studies, and so current findings should be regarded as provisional.

DNA copy number variations

DNA copy number variations (CNVs) are structurally variant regions in which copy number differences have been observed between two or more genomes. Defined as being larger than 1 kb in size, CNVs can involve gains or losses of genomic DNA, which may not be visible by standard G-banding karyotyping. These structural variants can alter the transcription of genes by altering dosage or by disrupting proximal or distant regulatory regions, as has been shown globally in healthy humans. Specific disease-associated CNV loci have also been identified, providing examples of how CNVs can alter cellular function (121). Genome-wide screening of populations affected by a specific disease could demonstrate the presence of CNVs related to disease incidence, clinical course and prognosis (122). Further refinement is required to assess CNVs as risk factors in complex diseases such as cancers. New technologies allow us to investigate CNVs in genome-wide scans and specific algorithms have been developed to determine CNV location and copy number (123).

Epigenetic regulation

Definitions of epigenetics vary in the literature, but as a minimum all include the concepts of molecular modifications to DNA and/or chromatin in the absence of any alteration to the underlying DNA sequence (124,125). Epigenetic alterations are increasingly being recognized for their roles in health and disease. The deregulation of epigenetic mechanisms is considered as a major cause of cancer, hereditary and neurodegenerative diseases.

DNA methylation patterns. DNA methylation of cytosine [5-methylcytosine (5mC)] is a normal epigenetic mechanism involved in controlling DNA structure, chromosome stability, the mobility of viral DNA-repeated elements (transposons, retrotransposons), gene imprinting and gene expression (126). The DNA methylation machinery includes families of methyl-binding proteins [*methyl-CpG binding domain protein (MBD)*, *steroid receptor RNA activator (SRA)* and zinc finger families (127)] conveying the biological signal of DNA methylation marks produced by families of DNA methyltransferases (DNMT1, -2, -3). The DNMTs catalyze the transfer of a methyl group from the universal methyl donor, S-adenosyl-L-methionine, mostly to the fifth position of cytosine in CpG dinucleotides. DNMTs often work cooperatively; expression of DNMT3B, usually low in somatic cells, tends to be increased in cancer and cooperates with DNMT1 in silencing tumor suppressor genes (128–130). The carcinogenic implications of genetic mutations or polymorphisms in DNMT

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genes, or genes encoding the one-carbon metabolism pathway enzymes (glycine-*N*-methyltransferase) or chromatin remodeling proteins, were recently reviewed (131). The removal of 5mC involves, as an intermediate step, the oxidation of 5mC into 5-hydroxymethylcytosine (5hmC). This reaction is catalyzed by the ten-eleven translocation (TET) family of enzymes. The TET enzymes are mutated in several types of cancer, affecting their activity and likely altering genomic 5hmC and 5mC patterns. It is generally believed that 5hmC may have a more specific role in regulating transcription, whereas 5mC might have additional roles in maintaining genomic integrity and transposon stability (132). The abundance of 5hmC is low in cancers, but reciprocal changes in abundance of 5hmC and 5mC during liver development as well as in response to the non-genotoxic carcinogen phenobarbital have been reported (133).

An important feature of cancer development and progression is the change in DNA methylation patterns, characterized by the hypermethylation of specific genes concurrently with an overall decrease in the abundance of 5mC. In various cancers, CpG islands (DNA regions with high frequency of CpG dinucleotides) located in promoter regions or elsewhere are frequently hypermethylated, which repress expression of tumor suppressor genes such as cyclin-dependent kinase inhibitor *P16*, *TP53* and the DNA repair gene *MGMT* (134) and allow cells to grow and divide uncontrollably. DNA methylation, independently or in association with methyl-binding domain proteins or repressive histone marks, prevents binding of DNA polymerases (135,136) and transcription factors (135,137). Changes in DNA methylation occur not only in CpG islands but also throughout the genome where they also contribute to gene expression regulation. In addition to repressing tumor suppressor gene expression, DNA methylation contributes to genome instability given that 5mC is a hot spot for both germ line and somatic point mutations, since 5mC can undergo spontaneous or enzymically driven deamination to thymine creating C > T transversion mutations [reviewed in (138–140)].

In particular, hypomethylation can lead to overexpression of oncogenes. Ehrlich *et al.* (141) indicated that a major contributor toward the overall or global DNA hypomethylation seen in most cancers is the loss of methylation in tandem and interspersed DNA repeats, occurring as a result of demethylation and not a preexisting hypomethylation in a cancer stem cell. Repetitive DNA sequences account for approximately 40% of the genome (142) and methylation of these sequences is required to maintain chromosomal stability.

Histone modification. At all phases of the cell cycle, the interactions between chromatin and DNA are constantly being remodeled, ensuring the normal functioning of DNA replication, gene transcription and DNA repair. To achieve these cellular functions, a series of coordinated enzyme reactions are required to create new histone molecules and histone marks and to assemble, disassemble and position new and old nucleosomes onto the DNA through replication-coupled or replication-independent pathways (143). The dynamic interaction between the chromatin and DNA is associated with a series of histone posttranslational modifications. Histones are nuclear globular proteins that can be covalently modified by acetylation, methylation, phosphorylation, glycosylation, sumoylation, ubiquitination and adenosine diphosphate ribosylation. These epigenetic marks constitute the 'histone code' (144) and contribute to the various functions of the chromatin. These histone modifications are reversible and performed by families of enzymes. Histone acetyltransferases, using acetyl-coenzyme A as the donor, add acetyl groups mainly to lysine residues of H3 and H4, which then lose a positive charge, reduce attraction to DNA and usually increase gene transcriptional activity. Histone acetylation is a marker of active genes. Acetyl groups are removed by histone deacetylases (HDACs), which then favor a contraction of the chromatin and gene silencing. Methyl groups can be added by histone methyltransferases, whereas histone demethylases remove methyl groups from arginine and lysine residues. Histone methylation can inhibit or increase gene expression depending on the amino acid position that is modified. Posttranslational modifications of histones have emerged as key regulators of genome integrity. Mutations in histone-modifying genes and in histone genes have been associated with tumorigenesis (131,145).

Epigenetic regulation by micro RNAs. Micro RNAs (miRNAs) comprise a large family of non-coding single-stranded RNA molecules of approximately 19–22 nucleotides in length. Recognizing the first 2–8 nucleotides of the 3′-untranslated region of their mRNA targets, miRNAs induce the target's degradation and downregulate gene expression at the posttranscriptional level (146). In mammals, miRNAs are predicted to control the activity of 30–50% of all protein-coding genes, which make their biogenesis and function a serious player in cell fate decisions and many critical biological events (147,148). It is generally believed that alterations in miRNA expression are the rule in human cancers. Several studies indicate that miRNAs can function as oncogenes or tumor suppressor genes (149), since they may be involved in the control of cell proliferation, inflammation, apoptosis, chromatin structure, genome instability (150), DNA damage response and DNA repair processes (151,152).

DNA methylation is known to regulate the expression of some miRNAs (153,154). Cell lines deficient in DNMTs, or treated with DNMT inhibitors alone or in combination with HDAC inhibitor, restored expressions of some miRNAs acting as tumor suppressor genes (miRNAs that are likely silencing oncogenic genes). Activation of miRNAs that are likely silencing tumor suppressor genes was also demonstrated (153), as well as miRNAs that target HDAC (SIRT1/HDACIII) and methyltransferase (EZH2) [reviewed in (155)]. Accumulating evidence in humans and mice shows an association between deregulation of miRNAs and exposure to environmental chemicals (156–158).

Other epigenetic influences on genome stability include several other classes of non-coding RNA molecules such as long non-coding RNAs and P-element-induced wimpy testis (PIWI)-interacting RNAs (159,160), as well as chromatin insulator DNA/protein complexes involved in transcription regulation, intra- and interchromosomal interactions (161).

Minisatellites

Micro- or minisatellites are simple tandem repeats that are scattered over the human genome. Because of DNA slippage events, the number of oligonucleotide repeats within these microsatellites can be changed leading to a phenomenon that is called MSI. This genetic unstable situation can disrupt other non-coding regulatory sequences or inactivate tumor suppressor genes, thereby driving the carcinogenic outcome. The MSI phenomenon may occur frequently in genomes of cancer cells when there is a defect in the DNA mismatch repair (MMR) system. Typically, DNA MMR enzymes are proofreaders of replicating DNA and restore replicative errors after exogenous and oxidative DNA-damaging insults (70,71,162). MMR is engaged in enhanced

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apoptosis, p53 phosphorylation and cell cycle arrest. Obviously, impaired function of MMR enhances replicative errors and can be observed through the detection of MSI, and MSI is a hallmark of MMR dysfunction observed in colorectal and other malignancies (163–165). Although defective MMR genes are normally found in MSI-positive cancers, a puzzling observation was that several MSI-positive cancers do not display genetic or epigenetic defects in any known MMR genes. An epigenetic explanation for these cases was recently discovered that involved deficiencies in SET Domain Containing 2 (SETD2) activities resulting in less efficient MMR. SETD2 is the only histone methyltransferase that induces histone H3 trimethylation (H3K36me3) and this histone mark was found to be required to attract the MMR machinery to the chromatin (25). There is accumulating evidence to support the notion that the interrelationship between MSI and miRNA plays a key role in the pathogenesis of gastrointestinal cancer (166). Nowadays, high-throughput sequencing data will undoubtedly lead to detailed knowledge of MSI events in unstable cancer genomes and giving information of the evolutionary cancer process (167).

mtDNA content

An additional way to induce genome instability is at the level of mtDNA. Each human cell contains between several hundred and over a thousand mitochondria, each carrying 2–10 copies of mtDNA. mtDNA-encoded genes are involved in the production of proteins essential for cellular respiration and normal mitochondrial function. Mitochondria—which have no protective histones and diminished DNA repair capacity compared with nuclear DNA—are highly prone to damage (113). The damage is compensated for by replication of mtDNA molecules and an increase in the cellular mtDNAcn (114–116). The increase in mitochondrial mass and mtDNA content as a marker of mitochondrial damage and malfunctioning has been considered an early molecular event of human cells in response to endogenous or exogenous oxidative stress (117). mtDNAcn is correlated not only with the size and number of mitochondria, which have been shown to change under different energy demands, but also with different physiological or environmental conditions. Cells challenged with ROS have been shown to synthesize more copies of their mtDNA and to increase their mitochondrial abundance, compensating for damage and meeting the increased respiratory demand required for ROS clearance. mtDNAcn alterations have been associated with impaired apoptosis and subsequent increased cellular proliferation (118), as well as with nuclear DNA mutations due to aberrant mtDNA insertion into the nuclear genome (119). Evidence on environmental exposure and mtDNAcn has begun to accumulate, showing increased blood mtDNAcn within blood cells in relation to exposure to benzene (119,120), ambient particulate matter (121) and polycyclic aromatic hydrocarbons (PAHs) (122,123). Individuals with higher blood mtDNAcn at baseline have a higher risk of developing lung cancer (124).

Chronic low dose exposures to chemicals—some issues to consider

In the risk assessment procedure, low dose can refer to an estimated dose near the lower end of an observed dose–response curve (point of departure) from which a safe exposure level is calculated. This low dose (point of departure) can be based on various parameters of dose–response analyses, including (i) bench mark dose that involves dose–response modeling, (ii) no observed genotoxic effect levels or (iii) no observed effect level, the highest level of the chemical that does not induce any (toxic) effect during continuous prolonged exposure (168,169). In terms of risk of human cancer and public concern, 'low dose' refers to concentrations to which workers or the general population are exposed or to the concentrations of chemicals that can be measured in human tissues or fluids (170,171). However, at present, it is impossible to define clearly when an exposure should be considered to involve a 'low dose' that has no adverse effect. As discussed in the following paragraphs many parameters intervene, and what is a low dose depends on the particular circumstances and conditions characterizing the exposure and the target.

Considerations of low dose and chronic versus acute exposures

In the general population, people are exposed to various chemicals and carcinogens in a cumulative manner over their lifetimes, while occupational exposure, or specific behavior or lifestyle, generates exposures that are more acute. Deriving an understanding of the impact of these various exposure scenarios (low dose, chronic, acute) on cancer risk is important but remains a scientific challenge.

Some observations suggest that chronic exposure to a mutagen is more likely to result in cancer than an acute exposure to a similar total dose. Experiments with *N*-nitrosodiethylamine or *N*-nitrosodimethylamine on a very large number of rats showed that duration of exposure contributes more to the risk of cancer than intensity of exposure (172,173). In humans, the amount of DNA adducts in white blood cells of persons occupationally exposed to high concentrations of PAHs was relatively (i.e. per unit of dose measured in air) lower than in persons exposed to lower concentrations (174,175). Also in humans, chronic exposures to benzene and styrene appeared to induce a relatively stronger genotoxic effect at low dose (176,177). These observations and the principle that carcinogenesis involves sequential mutations over a period of time in a clone of initially transformed cells provide support for the hypothesis that chronic exposures might be more mutagenic than acute exposure to mutagens.

With regard to low dose effects of genotoxic carcinogens and radiation, some researchers have found controversial hormetic or protective effects (178). This has led to the hypothesis that exposure to low doses of genotoxic agents could reduce the risk of cancer. Indeed, many DNA repair enzymes are inducible by DNA damage (179,180), but many DNA repair mechanisms act at maximal intensity only after maximal induction, meaning that low dose exposure might not be able to trigger DNA repair enzymes (80,89,90). Environmental exposures of the general population are usually at low doses—and lower than the doses reported to result in an adaptive response associated with induction of DNA repair. Although several research groups claim to have evidence for the existence of a hormetic effect implying that a limited exposure to some genotoxic agents might protect health, Crump *et al.* (541) found little evidence in a comprehensive animal radiation database to support the hormesis hypothesis. In addition, there is overwhelming evidence, mainly from experimental work and from molecular epidemiology and to a lesser extent even from epidemiology, that exposures of low intensity do have harmful effects (181–186). Druckrey *et al.* (187) found indeed that if a dose of the carcinogenic alkylating agent diethyl nitrosamine is reduced, carcinomas of the liver and esophagus in rodents still occurred but required a longer time to develop. Haber *et al.* made similar observations [reviewed in (188)].

Finally, the types of DNA damage induced by environmental contaminants differ from those occurring naturally and

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thus can be more mutagenic than DNA damages induced endogenously, and this calls for improved environmental hygiene.

Susceptible (cell) populations

Different tissues and cell types exhibit varying DNA repair capacities and are therefore likely to differ in susceptibility to environmental exposures with respect to induction of mutations, changes in the epigenome and genome instability. There are distinct implications for cancer development as to whether genome instability or mutations are transmitted through the germ line and thus are present in all cells of the individual or are acquired later in life and thus are not omnipresent. Increased rates of congenital mutations or epigenetic dysregulation naturally lead to increased cancer risk later in life.

In stem cells, DNA damage that is not repaired can lead to mutation amplification or propagation through the processes of self-renewal and differentiation, respectively, whereas damage to postmitotic cells can affect mostly tissue homeostasis.

Germ cells

Germ line mutations can be inherited via the mother or the father, and any exposure or disruption of biological functions that leads to increased mutation rates in either of the parents may influence the susceptibility of the child to cancer. A dysregulated epigenome in offspring originating via the paternal or the maternal genome through reproduction will potentially influence genetic instability and cancer proneness of the offspring. There are indications that parental lifestyle can be associated with increased risk of childhood cancer, and the significance of the father's exposure may be particularly important (189–191). Increased minisatellite mutations in offspring were found to be associated with paternal lifestyle factors (192). Male germ cell types exhibit different repair characteristics compared with most somatic cell types (193–197). Unrepaired DNA adducts in sperm are transferred to the mouse zygote, and epigenetic mechanisms may be involved (198,199). It has been reported that new mutations arising in the germ line (*de novo* mutations) lead to a significant portion (~20%) of the genetic disorders occurring in infants (200,201). *De novo* mutations can arise both in the mother and in the father, but the types of mutations inherited from the mother are different from those from the father. This indicates that the kind of stress put on the parents is likely to give different outcomes with respect to genome instability and mutations in their offspring.

Stem cells

Stem cells differ markedly from somatic cells in how they cycle and cope with genotoxic insults.

Stem cells have the unique properties of self-renewal and potential to differentiate into various cell types. Embryonic stem cells, derived from the inner cell mass of a blastocyst stage embryo, are able to differentiate into any cell type, showing the hallmarks of pluripotency. Tissue-specific or adult stem cells are responsible for maintaining homeostasis by cell replacement or repair after tissue damage. Tissue-specific stem cells have been found in both regenerative (blood, skin, digestive tract) as well as in non-regenerative organs such as muscle or brain. Cancer stem cells are functionally similar to tissue-specific stem cells but with an aberrant self-renewal and differentiation capacity. They are found in a series of cancer types (leukemia, glioblastoma, breast and skin cancers) as highly clonogenic cells.

The importance of the maintenance of genomic integrity in stem cells, however, is higher compared with non-stem cells for different reasons. From a developmental perspective, it is clear that genetic damage generated and not repaired in stem cells could be transmitted to the progeny with severe consequences for the developing embryo or regenerating tissue. Chromosomal abnormalities, such as aneuploidy, can function as a diversifying agent. For example, 33% of mitotic neural progenitor cells are characterized by aneuploidy, and some of these are able to generate mature aneuploid neurons. It is thought that this aneuploidy leads to a higher degree of variability and to uniqueness (202,203).

Mutation frequencies are generally much lower in stem cells compared with somatic cells, for example, 100–1000 times lower in mouse embryonic stem cells compared with embryonic fibroblasts (204). This more stringent maintenance of genomic integrity in stem cells is the consequence of differences in cell cycle regulation and increased DNA repair capacity (205). Besides differences in cell cycle checkpoints and repair, two other ways to deal with DNA damage for embryonic stem cells are the induction of apoptosis or the process of differentiation in order to avoid passing mutations to their progeny.

In utero exposure

Defined point of departures should be interpreted with care, since exposures that had virtually no detrimental effect on a mature organism have been shown to cause very serious adverse effects on the developing fetus [reviewed in (206)]. Moreover, environmental chemical studies showed that exposures during development at low doses did not cause any 'acute' teratogenic end points, although dysfunctions and diseases showed up later in life. Interestingly, for certain tissues and organs (e.g. brain, lungs and immune system), the critical 'windows' of developmental vulnerability may continue beyond the *in utero* stage through the neonatal period and perhaps even into puberty, thereby extending the period of increased vulnerability to adverse effects from environmental chemical exposures. Exposure to chemicals *in utero* can have direct effects on the genome integrity (5,207–209) though epigenetics, as a modifier of the mechanisms maintaining genome integrity, has become the cornerstone of the 'Developmental Origins of Health and Disease' hypothesis, which points to epigenetic regulation as the likely mechanism behind the environment-driven epidemic of non-communicable disease such as many cancers (210–212). Epigenetics may thus provide a new tool for understanding mechanisms underlying well-recognized gene–environment interactions and their role in carcinogenesis.

Chemical disruptors and their effect on biological target sites

The term 'chemical disruptor' in this section refers to a chemical within the environment that is able to affect the integrity of the genome. For example, with regard to carcinogenesis, a chemical might be selective for its chemical reactivity with DNA, as opposed to another chemical that might be selective for interference with microtubule polymerization and therefore disrupt the separation of replicated chromosomes during cell division. In Table 1, we summarize chemicals present in the environment that can selectively affect a specific pathway that when disrupted can induce genome instability. The selection of chemicals was based on criteria preestablished by the non-governmental organization Getting to Know Cancer (gettingtoknowcancer.org), which expressed interest in fostering a better understanding of the potential carcinogenicity of environmental chemicals and invited scientists to prepare this review. The guiding criteria for chemical selection were (i) to limit the number of chemicals to 10; (ii) to select, rather than chemicals

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that directly attack the DNA or cause mutations (which have been studied thoroughly for decades), chemicals that create indiscriminate damage by affecting, for example, DNA repair pathways, epigenetic pathways or mitochondrial function (indirect effects that have only recently received attention); (iii) to focus on chemicals that are not currently known as carcinogenic to humans (class 1; according to IARC), but which have properties that place them into areas of concern; (iv) the selected chemicals should be ubiquitous in the environment. It has not been our aim in this review to give a full toxicological evaluation of the selected chemicals (Table 1), but only to highlight the pathways (or biological targets) through which they can affect genome stability. For each of the biological target sites, one chemical was selected (identified in bold characters in Table 1) that could be regarded as a 'prototypical' chemical to act via this pathway to promote genome instability.

Heavy metals

Toxic metals are important environmental hazards because of their wide distribution and long persistence leading to accumulation in biological systems. Mercury (inorganic, IARC group 3), lead (inorganic, IARC 2A), nickel (IARC 1 for Ni compounds and alloys and 2B for metallic nickel) and cobalt (metal with or without tungsten carbide (WC), respectively, IARC 2A and 2B)—as well as the carcinogenic compounds cadmium and arsenic—have been reported to inhibit DNA repair. Even though their DNA-damaging potentials are rather weak, they interfere with the nucleotide and BER at low, non-cytotoxic concentrations (213–215). Various steps of the repair process can be affected. A mechanism of action (studied *in vitro* in PM2 bacteriophage DNA) is alteration of the binding activity of zinc finger proteins through displacement of Zn(II) by those heavy metals at levels just above 1 mM for lead, nickel and cobalt, but at doses as low as 50 nM for mercury (213–215). DNA repair can also be inhibited by the production of ROS (247). Toxic metals have been shown to affect DNA methylation and DNMTs and therefore influence gene transcription in animal and human studies (248). Prenatal arsenic exposure was recently shown to be related to 5mC alterations in human cord blood (249). In workers, nickel (alongside chromium and arsenic) was also shown to be capable of inducing posttranslational histone modifications by affecting the enzymes that modulate them (223–225). Changes in miRNA expression levels were associated with exposures to epidemiologically relevant concentrations of an arsenic, cadmium and lead mixture [2 µM $NaAsO_2$, 2.4 µM $CdCl_2$ and 4.8 µM $Pb(Pb(C_2H_3O_2)_2 \cdot 3H_2O]$ in a mouse fibroblast cell line (250).

Metal alloy dusts

Combinations of WC and cobalt or nickel or iron are used to improve the robustness of hard metals. Cobalt with WC is probably carcinogenic to humans (group 2A), whereas without WC it is classified as possibly carcinogenic (group 2B). Exposure to hard metal alloy dusts has been associated with lung cancer (251,252). Also tungsten metal alloys were found to transform a human osteoblast-like cell line, and this was in part considered to be due to direct DNA damage (229). Intratracheal instillation of these alloys (92% tungsten/5% nickel/3% cobalt and 92% tungsten/5% nickel/3% iron) has been linked to increased ROS production, induction of genes associated with oxidative stress and metabolic stress in rats, which contribute to lung injury through increased inflammation and oxidative stress (230). In type II rat pneumocytes, single intratracheal instillation of WC-Co (6.3% cobalt, 84% tungsten and 5.4% carbon) induced single-strand breaks and chromosome mutations (micronuclei) (253). Interestingly, Lombaert *et al.* (231) described a possible hypoxia-inducible factor 1 signaling cascade after *in vitro* exposure to the alloy WC-Co mixture (94% tungsten and 6% cobalt), in human peripheral blood mononucleated cells, whereas individually WC and cobalt chloride did not induce the cascade. The authors discussed the possibility that the effect on hypoxia-inducible factor 1 responsive genes could increase the carcinogenic properties of the mixture (231).

Acrylamide

Acrylamide can be ingested, inhaled (e.g. in tobacco smoke) or absorbed through the skin. Fried, starchy foods are the most prominent sources of exposure. The reaction between asparagine and fructose typically produces the most acrylamide in foods from plant sources. Hemoglobin adducts present a reliable short-term measurement of acrylamide and glycidamide (CYP2E1-mediated DNA-reactive metabolite of acrylamide) exposure (235,254). It is well established that the glycidamide metabolism product reacts readily with DNA, which has been shown to be a major route for its genotoxicity. Already in the 1990s, acrylamide was shown to be positive in the Muta Mouse transgenic mutation assay (255). Clastogenic effects were noted in a number of tests for genotoxicity and assays for germ cell damage. In 1994, the compound was classified as a probable human carcinogen (IARC 2A). The US National Centre for Toxicology Research has conducted a lot of work on the toxicity and carcinogenicity of the compound and recently showed clear evidence of carcinogenicity in a 2 year study in rats and mice (256). There are epidemiological indications that common dietary acrylamide concentrations might cause postmenopausal endometrial and ovarian cancers, but epidemiological studies are not conclusive as they might be confounded by the fact that the exposure is population-wide (236). Acrylamide was included in Table 1 since (apart from DNA adduct formation), alternative mechanisms causing indirect acrylamide carcinogenicity are described. Indeed, effects on kinesin proteins (expressed in bacteria using recombinant DNA techniques) could explain some of the genotoxic effects of acrylamide. These proteins form the spindle fibers in the nucleus that function in the separation of chromosomes during cell division (216,237). Other mechanisms underlying acrylamide-induced carcinogenesis or nerve toxicity are related to an affinity for sulfhydryl groups on proteins. Binding of the sulfhydryl groups could inactive proteins/enzymes involved in DNA repair and other critical cell functions (216).

Bisphenol A

Bisphenol A (BPA) is used to produce polycarbonate plastics which, due to their properties of transparency and hardness, are often used in food and drink packaging. The compound has been detected in body tissues and urine in most western populations. United States Environmental Protection Agency, the US Food and Drug Administration, the European Food Safety Authority and the Japanese Institute of Advanced Industrial Science and Technology have concluded that human exposure to BPA is below safe exposure levels (257–259). BPA is not classified as a human carcinogen (IARC group 3). However, there is much evidence of BPA being able to transform cells *in vitro* and acting as an aneugen by interfering with microtubule assembly and therefore with the function of the spindle apparatus during mitosis in human fibroblasts *in vitro* (260). Although apparently high concentrations of BPA (250 µM and more) were required in the culture medium, it was indicated that it was unknown how much BPA in the medium was protein-bound and how much reached the cells (cytotoxicity was only observed above 400 µM). BPA has been found to have a threshold effect with regard to its action as an aneugen (261). BPA can also

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Table 1. Selected pathways through which chemical disruptors can affect genome stability

Biological target site	Affected part of pathway	Mode of action	Chemical disruptor[a] (IARC group)	Human exposure	References
DNA repair pathways	Repair enzymes	Binding of repair enzymes (zinc finger proteins) resulting in dysfunctional DNA repair	Metals: **Lead** (2A), nickel (metallic: 2B, compounds + alloys: 1), cobalt (2B, 2A with WC), mercury (3)	Ingestion for contaminated crops and water	(213–215)
		Binding of the sulfhydryl groups could inactivate proteins/enzymes involved in DNA repair and other critical cell functions	**Acrylamide** (2A)	Ingestion of heated, fried and baked food	(216)
Epigenetic pathways	DNA methylation	Disruption of DNA methylation patterns via affecting DNMTs	Bisphenol A (3)	Leaking from plastics into food and water	(217,218)
			SiO_2-NP (not evaluated by IARC)	Leaking from, e.g., rubber, plastics, paints, glass, steel, batteries, adhesives as well as in cosmetics	(219)
		Possibly react with thiol functional groups of free cysteine residues in catalytic center of DNMTs	**Quinones** (3)	Drinking water disinfection by-products	(220)
	Histone modifications	Disruption of histone acetylation; affect histone acetylases and deacetylases	Bisphenol A (3)	Leaking from plastics into food and water	(221)
			Quantum dot-NP (not evaluated by IARC)	Used for optical (e.g. light emission diode [LED] displays), electronic and computational applications	222)
		Affecting enzymes that modulate post-translational histone modification	**Nickel** (metallic 2B, compounds + alloys: 1)	Primarily via oral intake, as a contaminant in drinking water or as either a constituent or contaminant of foods, including chocolate, nuts and grains	(223–225)
	miRNA	Disturbed binding to mRNA	**Bisphenol A** (3)	Leaking from plastics into food and water	(226)
		Changes in miRNA expression	Metals: nickel (metallic 2B, compounds + alloys: 1), titanium (not evaluated by IARC)	Ingestion for contaminated crops and water	(227)
			Gold (Au) NPs (not classified)	Used in electronics, paint, as sensors in food and as an antibiotic, antifungal and antimicrobial in plastics, coating and textile	(228)
DNA damage signaling	Redox signaling	Disruption of redox signaling involving *Nrf*, *NF-κB*, *early growth response (EGR)*, etc.	**Alloy particles**: tungsten/nickel/cobalt (2A, 2B)	Inhalation	(229–231)
			Bisphenol A (3)	Leaking from plastics into food and water	(232)
Mitochondrial function	Mitochondrial respiration	Decreased Nicotinamide adenine dinucleotide (NADH) levels and impairs mitochondrial membrane potential and mitochondrial respiration accompanied by ROS generation	**Titanium dioxide NPs** (2B)	Used as additives in many food products and cosmetics; also present in paints, plastics (e.g. toys and food packages), paper, clothes, etc.	(233)
		Activation of the mitochondrial germ cell apoptotic pathway: *B-cell CLL/lymphoma 2 (Bcl-2)* family and caspases in testis	Bisphenol A (3)	Leaking from plastics into food and water	(234)
Cell cycle/cell division	Spindle defects	Spindle defects leading to formation of micronuclei	Acrylamide (2A)	Ingestion of heated, fried and baked food	(235–237)
			Benomyl (+ metabolite Carbendazim) (2B)	Ingestion from residues in crops	(238–240)
			Bisphenol A (3)	Leaking from plastics into food and water	(241)
			Carbon nanotubes (to be evaluated by IARC)	Used in many consumer and industrial products	(242–244)
Telomeres	Telomere loss	Defect in telomere maintenance	**Lead** (2A)	Ingestion for contaminated crops and water	(245,246)
			Carbon black (2B)	Inhalation	(246)

[a]Chemicals in bold are the once that could be regarded as 'prototypical' chemical to act via this pathway to induce genome instability.

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bind the estrogen receptor and elicit unwanted effects such as induced growth of MCF-7 human breast cancer cells. When the estrogen receptor is the selective target, there is evidence that BPA can induce non-monotonic dose–response curves. The endocrine system is primed to detect and respond to low levels of hormones. Moderate changes within the low dose concentrations of estrogen in women can have large effects because of the availability of estrogen receptors (262,263). It was predicted via *in vitro* human cell models that an estrogenic endocrine disruptor such as BPA would be biologically active in, for example, the fetus if the estrogenic activity of the free chemical in blood was equivalent to an increase in free estradiol of only 0.1 pg/ml (255).

In utero and neonatal exposure in rodents to low doses of BPA and/or phthalates di (2-ethylhexyl) phthalate (DEHP)/monoethylhexyl phthalate (MEHP) and butyl benzyl phthalate (BBP)/dibutyl phthalate (DBP)/ monobutyl phthalate (MBP)) may cause aberrant changes in DNA methylation at CpG islands near gene promoter regions (217,218), histone modifications (acetylation, methylation, phosphorylation, ubiquitination, sumoylation and adenosine diphosphate ribosylation) (221) and expression of non-coding RNAs, including miRNAs in human placental cell lines *in vitro* (226). Such epigenetic marks can induce up/down alterations in gene expression that may persist throughout a lifetime. These permanent changes can result in adverse health effects such as neural and immune disorders, infertility and late-onset complex diseases (cancers and diabetes). The transient exposure of gestating female rats to BPA and phthalates (1% of lethal dose 50% (LD50)) was further shown to cause a transgenerational differential DNA methylation in the F3 generation sperm epigenome (264,265).

Quinones

Halobenzoquinones—such as 2,6-dichloro-1,4-benzoquinone, 2,6-dichloro-3-methyl-1,4-benzoquinone and 2,6-dibromo-1,4-benzoquinone (among other quinones)—have recently been reported as disinfection by-products in chlorinated drinking water (266–268). Quinones are electrophilic compounds, known to react with proteins and DNA to form adducts (269). Animal studies provided inadequate evidence of cancer (IARC group 3). More recently, Wang *et al.* (220) showed, via an *in vitro* inhibition reaction, reduced DNA methylation by quinones (some of them starting already at 0.1 µM concentration) probably due to inhibition of DNMT. These electrophilic chemicals most probably interact with functional thiol groups via Michael-type addition, causing free cysteine residues of DNMTs to be modified (220). In this context, another interesting target that could explain active demethylation involves the TET family of Fe^{2+}- and α-ketoglutarate-dependent 5mC deoxygenases. That mechanism was discussed for benzene and its metabolite hydroquinone as a possible pathway for decreased methylation (besides inhibition of the DNMTs) in an HEK293 cell line (at a concentration of 60 µM, with cytotoxicity occurring at 100 µM hydroquinone). It was suggested that an increased conversion of 5mC to 5hmC catalyzed by TET1, followed by deamination to 5hmU and subsequent removal by BER, could result in an unmethylated cytosine (270). This mechanism might be shared by other xenobiotics that increase ROS.

Benomyl

The fungicide benomyl is metabolized to carbendazim; both are classed as possible human carcinogens (IARC group 2B). The route of exposure is most likely ingestion via residues in crops. Benomyl has an aneugenic mechanism of action, disrupting the microtubules involved in the function of the spindle apparatus during cell division, leading to production of micronuclei. Benomyl and its metabolite have both been associated with hepatocellular tumors in male and female rodents (238,239). Also threshold concentrations (of 3.2–4.1 mM) have been identified based on *in vitro* and animal studies for these compounds, with regard to non-disjunction and chromosome loss (271). Considering non-disjunction in the human chromosome 17, after exposure *in vitro*, there is evidence of significant response only above an exposure of 1.1 µg/ml. However, it was found to be among seven chemicals that, when combined at concentrations below their individual threshold, showed a response *in vitro* (272). Induction of micronuclei was observed, which could be explained by the additive effect of chemicals with a similar mechanism of action.

Nano-sized particles

During the past decade, industries related with cosmetics, biomedical compounds, textiles, food, plastics and paints, among others, have been taking advantage of the unique characteristics of nano-sized materials. Consequently, human exposure to these types of materials has increased not only in a conscious way but also passively by the leakage of nanomaterials from different objects. Their physicochemical and biological characteristics cause them potentially to disrupt the normal function of mammalian cells and increase the toxicity and genotoxicity of some materials. Available literature about nanomaterial genotoxicity illustrates the complexity of identifying and understanding the hazard of NP in relation to human health. Not only is the chemical composition of nanomaterials responsible for their genotoxicity but also shape, specific surface area, size, size distribution and zeta potential determine the effects of these materials on the genome. Moreover, there is still a debate about the suitability of standard genotoxicity assays for studying the effects of nanomaterials (273,274).

Nanoparticles.

In addition to affecting mitochondrial respiration, NPs can induce genome instability via different mechanisms of action: (i) $ZnFe_2O_4$-NP (275), SiO_2-NP (276,277), CuO-NP (278) and TiO_2-NP (233) were shown to impair mitochondrial function *in vitro*; (ii) Ag-NP (279,280), ZnO-NP (281) and carbon black-NP (282) induced mitochondrial-related apoptosis *in vitro*; (iii) decreased DNA repair was observed due to exposure to TiO_2-NP (283), Ag-NP (280), SiO_2-NP (219) and Au-NP (284) *in vitro*; (iv) many NPs induce micronuclei *in vitro* and *in vivo* [e.g. CuO-, Fe_3O_4-, Fe_2O_3-, TiO_2-, as well as Ag-NP *in vivo* (285), and SiO_2-NP (286) *in vitro*]; (v) exposure to quantum dot-NP in mammalian cell lines can lead to hypoacetylation of histones (222); (vi) DNA methylation can be disrupted by SiO_2-NP in mammalian cell lines (219) and (vii) miRNA can be upregulated due to Au-NP, in the fetus of administered mice (228). Other mechanisms can also be responsible for the indirect genotoxicity of nanomaterials; they produce inflammation and alteration of the antioxidant defenses that can also lead to genome instability. Synergistic genotoxicity between NP and other chemical compounds has also been described in a mammalian cell line (287).

The majority of the studies are performed after an acute exposure and in *in vitro* systems. Their material diversity, and the lack of chronic exposure studies and *in vivo* studies using relevant concentrations of NP, makes it difficult to assess the real adverse effect of NP on human health, including their effect on the genome. Adaptive responses *in vitro* (288) as well as induction of genome instability *in vitro* (289) and *in vivo* (290) have been described in long-term studies.

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Carbon nanotubes.

Carbon nanotubes are a specific type of nano-sized particles, which are used in many consumer and industrial products, including electronic and drug-delivery products, protective clothing, sports equipment and space exploration (242). Preliminary information indicates that carbon nanotubes may have a similar carcinogenic potential to other durable natural or man-made fibers. That is one of the reasons why IARC has placed carbon nanotubes on the list of compounds that need evaluation with high priority during 2015–19. Muller *et al.* (243) showed for the first time the genotoxic and mutagenic potential of carbon nanotubes in lung cells, both *in vivo* and *in vitro* at (sub)cytotoxic concentrations. The aneugenic effect induced in the MCF-7 human breast cancer carcinoma cell line was speculated to be due to physical interaction with components of the mitotic spindle during cell division or to interaction with proteins directly or indirectly involved in chromosome segregation (e.g. tubulin, actin) (243). Indeed, the size and physical properties of carbon nanotubes are strikingly similar to cellular microtubules. Recent publications describe mitotic spindle aberrations in cultured primary and immortalized human airway epithelial cells exposed to high and workplace relevant concentrations (i.e. at doses equivalent to 20 weeks of exposure at the permissible exposure limit for particulates not otherwise regulated). Sargent *et al.* (242) demonstrated fragmented centrosomes, disrupted mitotic spindles and aneuploidy chromosome number following 24–72h exposure to concentrations of carbon nanotubes that could be anticipated during workplace exposure. Interaction of the mitotic motors with carbon nanotubes or carbon nanotube/microtubule hybrids may result in incorporation into the mitotic spindle, which is highly associated with carcinogenesis (242,244).

Carbon black.

IARC classifies carbon black particles as possibly carcinogenic to humans (2B). According to the IARC carbon black is a generic term for a particulate form of elemental carbon manufactured by the vapor-phase pyrolysis and partial combustion of hydrocarbons. It is mainly used as reinforcing filler in tires and other rubber products. In plastics, paints and inks, carbon black is used as a color pigment. Carbon black is widely used as a model compound for assessment of the impact of pollutant diesel soot (black carbon, BC). The latter particles are a good indicator of adverse health effects from urban air pollution and are ubiquitous in the environment, alongside other air pollution particles (291). Oxidative stress induction is one of the plausible mechanisms leading to these particles' potential as a genome-destabilizing agent. Indeed, ROS formation has been shown to reduce nuclear as well as mitochondrial telomerase activity (292). Büchner *et al.* (292) showed a lower telomerase activity in endothelial and lung epithelial cells *in vitro* exposed to ultrafine carbon black using concentrations the vessels (1 μg/cm^2) and lung (10 μg/cm^2) are daily exposed to. McCracken *et al.* (293) reported in elderly men an association between long-term ambient BC concentrations (interquartile increase of 0.25 μg/m^3) at their residences and shorter telomeres. Environmental and occupational exposures to BC, and other traffic-related air pollutants, such as particulate matter, benzene and toluene, are associated with shorter telomere length (57). Reduction of telomere length by BC may be due to both: (i) an increased rate of hematopoetic stem cell replication for replenishing leukocytes needed in the inflammation process following BC exposure (accelerated senescing) and (ii) telomere loss per replication, caused by oxidative stress (57,293).

Cross talk between genomic instability and other hallmarks of cancer

Carcinogenicity of low dose exposures to chemical mixtures in any given tissue will likely depend upon simultaneous instigation of several important tumor promotion mechanisms and the disruption of several important defense mechanisms. Accordingly, we undertook a cross-validation activity to illustrate the effect the target pathways and/or the chemical disruptors discussed have on other cancer hallmarks (Tables 2 and 3). The literature search was performed within PubMed and Google using the following key words along with the compound/chemical of interest: genomic instability, mutation, genotoxicity, clastogenic, etc. Some of the prototype examples were included in the tables.

For example, mutation of tumor suppressor p53, an important guardian gene, not only promotes genomic instability but has also been shown to play a role in promoting chronic inflammation via sustained NF-κB activation and participation in tumor-promoting inflammation (524). Telomerase activity prevents telomere loss that can lead to genomic instability (see Caretakers of genome integrity). However, high telomerase activity is also associated with increased invasiveness and metastatic capability (another cancer hallmark) of colon, breast, gastric and liver cancers (407,408,525,526). It is also interesting to note that certain mechanisms that maintain genome stability can be pro-carcinogenic in some cases. The cell's primary defense mechanisms against mutagenic DNA damage are numerous DNA repair pathways. In the presence of DNA damage, the cell has two fates. Either damage will be repaired by repair pathways or the cell will undergo damage-induced apoptosis. Therefore, in a paradoxical manner, the same DNA repair pathways that are charged with preventing damage from being fixed to carcinogenic mutations may promote the survival of an initiated but damaged cell (297).

It is also useful to understand the cross talk that potential chemical disruptors of genomic stability processes have with other cancer hallmarks (Table 3). Interestingly, this has both pro- and anti-apoptotic effects on cells, depending on cell type. In promyelocytic leukemia and ovarian granulosa cells, BPA induced apoptosis (461,462) while it promoted survival and proliferation in breast epithelial cells (459,463). It is noteworthy that, although many of the compounds discussed in this review are pro-carcinogenic in their ability to promote genomic instability, they are simultaneously anticarcinogenic in their ability to promote apoptosis; for example, the metals lead, nickel, cobalt and mercury act in this way (411,420,430). Apoptosis is often independent of the genotoxic effects of the metal as is the case for nickel and mercury (430,439). However, most of them can also lead to sustained proliferation, another hallmark of cancer, by a wide variety of mechanisms, including induction of oncogenes, modification of miRNA signaling and hypoxia (414,424,432). The literature review performed in the preparation of these tables (although not exhaustive) suggests knowledge gaps particularly related to the possibility that chemical disruptors (Table 3) contribute to immune system evasion as well as tissue invasion and metastasis.

Overall, the cross-validation exercise demonstrated that many chemicals with documented effects on genomic stability simultaneously affect other cancer hallmark pathways, but a large portion of cross talk remains to be studied, which the cross-validation highlighted (Table 3). Additionally, the cross-validation of genomic instability target pathways demonstrated that there is some cross talk between the genomic

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instability targets and other cancer hallmark pathways (Table 2). Further study is required to determine the involvement of genomic instability target pathways in a few areas, including the tumor microenvironment and angiogenesis. Finally, we believe the cross-validation results have provided a valuable update concerning progress toward understanding the connections among these cancer pathways and environmental chemical disruptors.

Discussion and conclusion

Genome instability is an enabling condition that can lead to cancer development (1) and is defined as an increased tendency of the genome to acquire mutations. This review raised the hypothesis that in addition to known human carcinogens, exposure to low doses of other chemicals in our environment could contribute to carcinogenesis by indirectly affecting genome stability. The chemicals discussed here (Table 1) can promote genome instability by interfering with pathways such as DNA damage signaling (heavy metals, BPA), DNA repair (heavy metals, acrylamide), epigenetics (nano-sized particles, BPA, quinones, heavy metals), mitochondrial function (NPs, BPA), spindle apparatus during cell division (acrylamide, benomyl, BPA, NPs) or telomere integrity (heavy metals, carbon black).

Cancer evolves as the long-term result of multiple causal interactions between environmental and genetic/epigenetic factors (3). Cancer cell types acquire their characteristics with different strategies, timeframes and microenvironments (1). Assessing the relevance of an internal dose of genome-disrupting chemicals should be done in the context of the exposome, being the life-course environmental exposures from the prenatal period onward (527). In this context, it is relevant to point out that the impact of genome instability varies throughout the life span; in particular, germ cells as well as the fetus show increased sensitivities to genotoxic agents and moderate (*in utero*) exposures can be associated with significantly increased mutation rates or changes to the epigenome of the human offspring (528). Individual variations in genotype, phenotype and exposome history are critically important factors determining the effect of genetic disruptors. Furthermore, populations are also experiencing temporal changes in chemical exposure. The abundance of most of the classical persistent organic pollutants has been steadily declining for many years while some flame retardants and PAHs are emerging or leveling off in human biological biomatrices such as breast milk and blood (529), and in the environment (530). In the past, a proportion of the working population in industrialized countries was undoubtedly exposed to high concentrations of (mixtures of) well-characterized carcinogenic substances. At present, due to increasing awareness of health risks and usually with improved worker protections, a great majority of the population in modern western countries have never been exposed to such substances at the high doses of the past. In some industries and/or regions, however, exposure is still substantial. The modern public health problem associated with carcinogenic or tumor-promoting substances is getting even more complex considering potential low dose effects of chemicals present in our daily life.

Special attention should be paid to the effect of combined exposure that can, through co-carcinogenic effects, lead to a rise in cancer risk (531,532). An interesting epidemiological study showed that individuals with multiple types of DNA adducts can have a 10-fold greater risk of cancer (532), thus implying that exposure to a mixture of genotoxins might more likely induce cancer than single chemicals. Interactions between (metabolites of) chemicals can and do occur. Metals such as lead, arsenic, cadmium and chromium constitute a frequently occurring quaternary mixture at hazardous waste sites. This mixture was found in soil at 219 out of 1608 sites examined by US Agency for Toxic Substances and Disease Registry. No pertinent health effects are known, nor do physiologically based pharmacokinetic models exist for such a mixture. However, intermediate-duration dietary studies of binary and trinary mixtures of lead, arsenic and cadmium in rats indicated that subthreshold doses (below the Lowest Observable Effect Levels (LOELs) for the individual metals, i.e. 5–25 p.p.m.)—when administered in combination—resulted in effects not observed when exposed individually (211,212). Other examples of mixtures include metals and PAHs that are frequently encountered as co-contaminants, such as in airborne particulates. PAHs, such as benzo[a]pyrene, are oxidized by CYP families (most importantly in this case by CYP1 families) to become DNA reactive and mutagenic. Numerous metals (arsenic, mercury, lead, cadmium, chromium, copper, vanadium) exert toxicities through various mechanisms. However, these metals were shown to modulate *CYP* activities (through changes in mRNA, or protein abundance, or catalytic activity) in tissue and species-specific manner but generally reduced *CYP* catalytic activities in human cells leading to a reduction in PAH-induced DNA damage [reviewed in (533)]. Altogether these examples demonstrate that the toxicity of a mixture differs from the toxicity of its individual components, which creates scientific difficulties in the risk assessment of mixtures.

In the 20th century, cancer incidence (after correction for age) has increased worldwide (534–536) in parallel with the spread of western technology, pollution and consumer products (181,182,537) and some cancers can be regarded as diseases of affluence. Lifestyle factors including diet, smoking and lack of exercise probably contribute importantly to the risk of cancer in a large part of the population of the more developed countries, but exposure to carcinogens or co-carcinogens present in polluted air and drinking water, as well as in food, is also thought to contribute significantly. However, it is not easy to test for links between cancer outcomes and exposure to chemicals or chemical mixtures at low doses. A recent review of investigations into causes of geographical cancer clusters in the USA (538) found that out of over 400 clusters, only one was unequivocally attributed to an environmental cause; a pleural cancer cluster linked to asbestos-exposed shipyard workers (538). It is difficult to detect the carcinogenic effect on humans of a single low-dose chemical through classical epidemiology, as a relative risk of <1.5 or 2.0 can rarely be shown to be statistically significant. Furthermore, with the exception of some cancer-prone conditions such as leukoplakia or colon polyps, presently no early markers for the whole of the carcinogenesis process are available. As yet we have no means to assess the extent to which a process of carcinogenesis has advanced in an individual.

Nevertheless, the more sensitive approach of molecular epidemiology (6,8,9,11–14,185,539) has shown interesting associations between certain environmental exposures and early biomarkers related to carcinogenesis. Several biomarker assays monitoring genome instability (e.g. microsatellite mutations, DNA damage/repair assessment, miRNA, DNA methylation changes in promotor regions, mtDNAcn, telomere length) are used in biomonitoring of individuals exposed to carcinogens. However, in most (but not all) cases, these intermediate biomarkers—though they may show up as significant in case–control

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Table 2. Cross-validation of target pathways

Genome instability priority targets	Deregulated metabolism	Evasion of anti-growth signaling	Angiogenesis	Resistance to cell death	Immune system evasion	Replicative immortality	Sustained proliferative signaling	Tissue invasion and metastasis	Tumor-promoting inflammation	Tumor microenvironment
DNA repair pathways	+ (294)	+ (295)	– (296)	+ (297)	+ (298)	+ (299–301)	+ (302)	+/– (303–306)	– (307)	0
Epigenetic pathways: DNA methylation	+ (308–310)	+ (311)	0	+/– (312–314)	+ (315)	+ (316)	+ (317)	+ (318–321)	+ (322)	0
Epigenetic pathways: histone acetylation	+ (323–325)	+ (326)	0	– (327–329)	+ (330)	+ (331)	+ (317)	Acetylases: – (332,333); Deacetylases: + (332,334–337)	(338)	0
Epigenetic pathways: disturbed miRNA binding	+ (339,340)	+ent (341)	0	+/– (342–344)	+ (345)	+ (346,347)	+ (348)	+/– (349–354)	+ (322)	0
DNA damage signaling: disturbed by redox signaling (NF-κB, Nrf, *early growth response (EGR)*)	+ (355,356)	+ (357)	+ (358)	– (297)	+ (359)	0	+ (360)	+ (361–367)	+ (368)	+ (369)
Mitochondrial function	+ (370,371)	+ (372)	– (373)	– (374)	+ (375,376)	+/– (377–379)	+ (380)	Respiration: – (381–383); Bcl-2: + (384–389)	+ (390)	0
Cell cycle/cell division: spindle defect	+/–; + (391,392)	+ (393)	0	+ (394,395)	+ (396)	+ (397)	+ (398)	0	+ (399)	0
Telomere loss	+ (400)	– (401)	0	– (402–404)	+ (405)	– (299)	– (406)	+ (407–410)	+ (399)	0

Target pathways for genome instability were cross-validated for effects in other cancer hallmark pathways. Targets that were found to have opposing actions in a particular hallmark (i.e. anticarcinogenic) were indicated with '–', whereas targets that were found to have promoting actions in a particular hallmark (i.e. carcinogenic) were indicated with '+'. In instances where reports on relevant actions in other hallmarks were mixed (i.e. reports showing both pro-carcinogenic potential and anticarcinogenic potential), the symbol '+/–' was used. Finally, in instances where no literature support was found to document the relevance of a target in a particular aspect of cancer's biology, we documented this as '0'.

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Table 3. Cross-validation of disruptors

Genetic instability prototypical disruptors	Deregulated metabolism	Evasion of anti-growth signaling	Angiogenesis	Immune system evasion	Resistance to cell death	Replicative immortality	Sustained proliferative signaling	Tissue invasion and metastasis	Tumor-promoting inflammation	Tumor micro-environment
Lead	0	0	0	0	– (411–413)	+ (57,245)	+ (414)	0	+ (415,416)	0
Nickel	0	+ (417)	+ (418)	0	+/– (419,420)	– (421,422)	+ (423)	+ (424)	+ (420,425)	+ PMID: (426)
Cobalt	+ (427)	0	+ (428)	0	– (429,430)	0	+ (431)	+ (432)	+ (433)	– (434)
Mercury	+ (435,436)	0	+/– (437)	0	– (438–440)	0	+ (441)	0	+ (442)	– (443)
Acrylamide	+/– (216,444–446)	0	+ (447)	0	– (448,449); – or no effect (450)	0	+ (451)	0	+ (452,453)	0
Bisphenol A	+ (454–457)	+ (458)	+ (459)	0	+/– (458,460–462)	+ (463)	+ (232)	0	+ (465)	0
Quinones	+/– (466,467)	– (468)	– (469)	0	– (470–472)	– (473–478)	– (479,480)	– (481,482)	+ (269,483)	0
Tungsten	+/– (466,484)	0	0	0	– (485–487)	0	+ (229)	0	+ (230)	0
Paraquat	+ (488–490)	0	0	0	– (491–493)	0	– (494)	0	+ (494,495)	+ (496)
Titanium dioxide NPs	+/– (497–499)	0	+ (500)	0	– (282,501)	0	+ (502)	0	+ (503,504)	0
Benomyl	– (505)	0	– (506)	0	0	0	– (507)	0	+/– +/– (508,509)	0
Carbendazim	– (505)	0	0	0	0	0	– (507)	0	+/– +/– (510)	0
Triclosan	+/– (511,512,540)	0	– (515)	0	– (514)	0	+ (515)	0	+ (516,517)	0
Carbon black	+ (518)	0	+ (519)	0	– (282)	+ (57)	+ (520)	0	+ (521)	0

Disruptors of genomic stability were cross-validated for effects in other cancer hallmark pathways. Disruptors that were found to have opposing actions in a particular hallmark (i.e. anticarcinogenic) were indicated with '–', whereas disruptors that were found to have promoting actions in a particular hallmark (i.e. carcinogenic) were indicated with '+'. In instances where reports on relevant actions in other hallmarks were mixed (i.e. reports showing both pro-carcinogenic potential and anticarcinogenic potential), the symbol '+/–' was used. Finally, in instances where no literature support was found to document the relevance of a chemical in a particular aspect of cancer's biology, we documented this as '0'.

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studies—have yet to be tested in prospective trials with clinical end points, to validate them as indicators of cancer risk.

Although epidemiological links between exposure to environmental chemicals and carcinogenesis are difficult to disclose, it is accepted that exposure to chemical mixtures and lifestyle factors can contribute to genome instability (530,538). A complex mixture of hundreds of environmental chemicals are present in the human body. Indeed, the Fourth National Report on Human Exposure-2009 provides blood or urinary exposure data for 212 environmental chemicals in the US population, with updated tables in 2013 for 151 chemicals (http://www.cdc.gov/exposurereport). Together with physical and biological agents, these chemicals might disturb the biological processes described in this special issue and contribute to the risk of developing cancers. Cancer can be viewed as resulting from an increase in entropy in the complex biological systems of the human body. Therefore, the implementation of a new form of hygiene, the physical chemical hygiene, comprising a reduction of exposure to chemical mixtures, among other genome-destabilizing agents, might well be a necessary condition for an effective prevention of cancer.

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