Lung Cancer: Mechanisms of Carcinogenesis

10

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Occupational lung cancer • Pulmonary carcinogenesis • Asbestos carcinogenesis • Oxygen radical damage • Chromosomal aberrations • Epigenetic changes • Carcinogenic metals

Introduction

Inhaled carcinogenic chemicals, mineral fibers and particulates, and carcinogenic metals are the most significant occupational causes of lung cancer. The gases, fumes, and particulates in industrial environments form complex mixtures, the carcinogenic potential of which may differ from that of each component separately. Particulate matter can absorb chemicals on its surface, which is thought to enhance the deposition of chemicals in the lung, their penetration into lung cells, and carcinogenic action. Personal or involuntary tobacco smoking complicates the exposures even further, since tobacco smoke is also a complex mixture containing carcinogenic agents in chemical and particulate forms.

The carcinogenicity of inhaled substances is influenced not only by their chemical composition but also by their retention and biopersistence in the lung. The pulmonary deposition and clearance of inhaled particles and fibers are dependent on particle size and dimension. Particles of 10 μm or more in diameter are deposited in the upper airways, whereas those around 1 μm or less in diameter are most effectively retained in the alveolar lung. Fibrous particles such as asbestos fibers are exceptional in their deposition and clearance, and asbestos fibers up to over 100 μm in length can be found in lung tissue. Inhaled particles and fibers are cleared from the lungs via lymphatics and mucociliary transport. Poorly soluble particles and fibers, which are retained in the lung, form a constant source of toxic damage.

This chapter reviews the carcinogenic mechanisms of the most significant pulmonary carcinogens. For more detailed information, we refer the reader to several recent comprehensive reviews cited in this chapter.

Asbestos

Occupational asbestos exposure and its clinical presentations have been described in Chapters 9, 14, and 15. Asbestos toxicity and carcinogenesis have been studied in a range of experimental settings, and several studies have shown that asbestos can induce malignant transformation in both murine and human cells [1]. Nevertheless, the exact molecular mechanism behind asbestos-related carcinogenesis is still unresolved. It is thought to be very complex, probably involving several parallel pathways (reviewed in [2]. Here, we discuss the specific mechanisms associated with asbestos-induced lung carcinogenesis. Chapter 17 includes a detailed discussion on the carcinogenic mechanisms in mesothelial cells following asbestos exposure. Different mechanisms may dominate in different cell types, and the sensitivity of cells to fibers may differ. Indeed the mesothelial Met5A cell line has been shown to be more sensitive to asbestos exposures than lung cells [3]. It has been

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proposed that this difference in sensitivity lies in the balance between oxidant and antioxidant levels (e.g., glutathione), which seem to be different in epithelial and mesothelial cells [3]. Nevertheless, some mechanisms may be similar or even the same, and it is important to compare the effects of asbestos fibers in different cell types. The molecular mechanisms involved in the carcinogenesis of specific cells may lead to the discovery of clinically useful molecular markers specifically associated with asbestos exposure in lung cancer. These markers are discussed in detail in Chapter 12.

Toxicity and Carcinogenicity of Asbestos Fibers

The genotoxic and carcinogenic effects of asbestos depend largely on the fiber's chemical composition and structure as well as the cell environment [4]. Several experimental studies have shown that the longer the fiber, the more carcinogenic it is per se [5]. However, many researchers argue that fibers of all lengths induce pathological responses and no type of asbestos fiber should be considered noncarcinogenic, based simply on its length [6]. On an epidemiological basis, it has been difficult if not impossible to establish such a hypothesis, since asbestos workers are often exposed to a mixture of different fiber types and sizes [7]. Amphiboles are thought to be more pathogenic in the human body compared to chrysotile, due to the metals they contain, the fiber structure, and their biopersistence. In contrast to chrysotile asbestos, which becomes fragmented and cleared from the lungs, amphiboles are considered to be totally insoluble in the human lung [8, 9]. It has been estimated that chrysotile fibers are needed at several hundred times the levels of amphibole fibers to induce a similar risk of malignancy (reviewed in [10]. On the other hand, there is considerable pathological as well as experimental evidence that also chrysotile is highly carcinogenic [11–13]; in fact, it has been established that chrysotile is as potent as the amphibole crocidolite, per fiber, in its ability to cause lung cancer, even though it is two to four times less potent in evoking mesothelioma [14] (see Chapter 17). However, for mesothelioma to develop, the fibers need to migrate to the pleural or peritoneal linings, while lung cancer development can be considered more direct, since they are "available" directly after inhalation. Thus, it is possible that the more efficient clearance of chrysotile is associated with its lower potency for causing mesothelioma compared to lung cancer.

Mechanisms of Asbestos Carcinogenesis

Oxidative Stress and Inflammation

Asbestos fibers enter the lungs through inhalation. In the bronchi and alveolar spaces, the fibers are surrounded by bronchoalveolar macrophages (BAM), which deposit an iron-protein coating around the fibers. These are then referred to as asbestos bodies. However, due to the larger size of the fibers compared to that of the BAM, the so-called frustrated phagocytosis may take place, leading to the elevated release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) as well as digestive enzymes, proteases, and chemokines/cytokines [4, 15]. Amphibole fibers contain high levels of associated mono-, di-, and trivalent metals such as iron, and it has also been proposed that asbestos is toxic by the particular way iron is bound to the fiber's surface, enabling the generation of ROS and RNS [16, 17].

Asbestos-related carcinogenic pathways are shown in Fig. 10.1. In addition to the generation of ROS and RNS, the main mechanisms behind the toxic effects of asbestos are thought to be alterations in mitochondrial function, mechanical disturbance of cell cycle progression, and the activation of several signal transduction pathways (reviewed in [4, 18, 19]) (see Chapter 17 for a more detailed discussion). Many of these effects are due to the triggering of universal cellular responses, induced by several types of cytotoxic substances. Interestingly, however, mitochondrial metabolism and ROS production appear to be necessary for *KRAS*-induced tumorigenesis in mice, and asbestos is indeed closely associated with mitochondrial dysfunction, which in turn is related to the inflammatory effects of asbestos (reviewed in [2]. Some, [20, 21] but not all, studies [22] have shown *KRAS* mutations to be associated with asbestos exposure in lung cancer (see Chapter 12 for a more detailed description).

Emerging evidence has also indicated that both *TP53* mutations and Myc-induced oncogenic transformation are dependent on mitochondrial dysfunction and ROS production (reviewed in [23], and these have both been implied in asbestos-related lung carcinogenesis [24]; reviewed in [2]). Thus, these mutations may be a consequence of the inflammatory effects associated with asbestos exposure. Lung cancer in general is considered an inflammation-associated cancer (reviewed in [23]. Cancer-related inflammation has been shown to vary between tumor types, and the prolonged inflammation induced by biopersistent fibers may have specific features. Thus, it is important to clarify the specific changes related to certain types of exposures, since this variation could possibly be used in cancer management (e.g., diagnosis and treatment) (reviewed in [23]).

Several genes involved in inflammation-associated expression pathways, such as those in the *TNFα/NF-κB* pathway, have proved to be deregulated in asbestos-related lung cancer. For example, TNFα, an inflammatory cytokine, has been shown to be activated in macrophages after asbestos exposure in vitro [25]. TNFα induces interleukin 8 (*IL8*) expression in macrophages, which attracts neutrophils that in turn release ROS and RNS. This leads to a feedback loop between ROS generation and increased *TNFα* expression, resulting in increased DNA damage [26] and consequently possibly increased mutations in critical genes, such as *KRAS* and *TP53*, as described above.

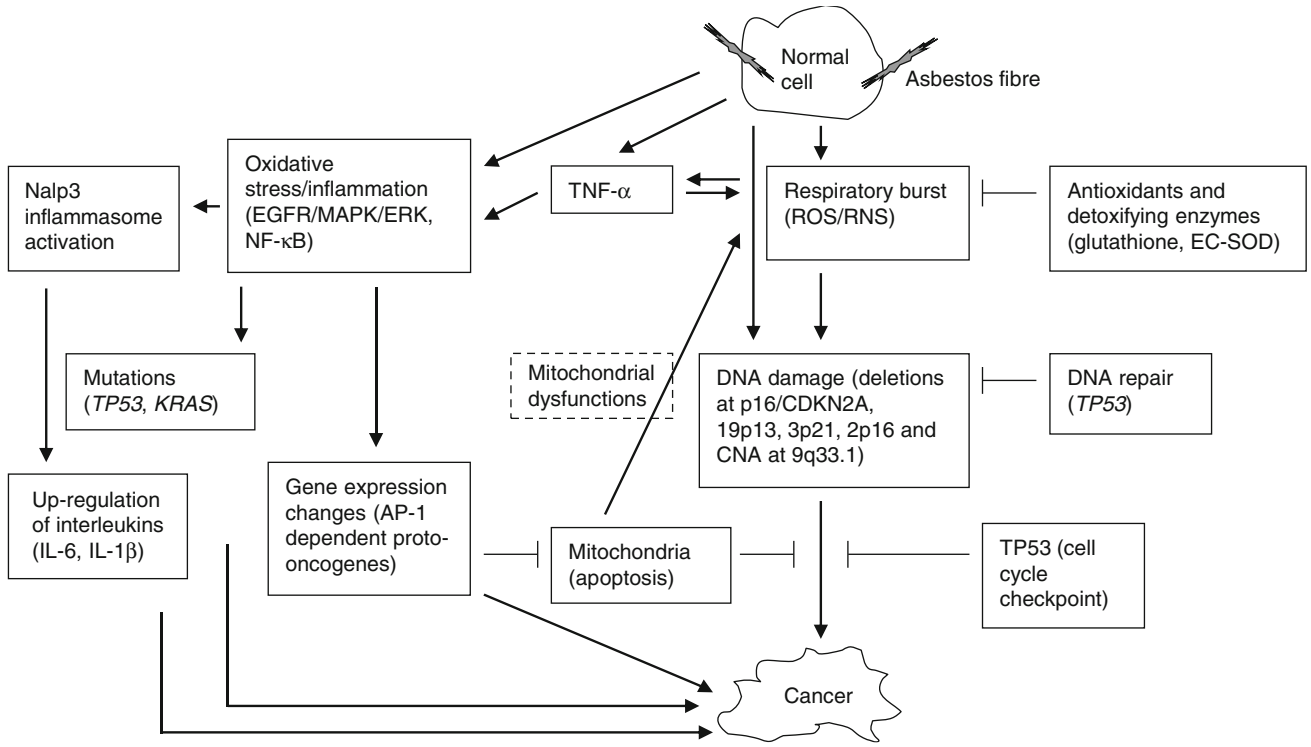

Fig. 10.1 Asbestos-related carcinogenic pathways in the lung (Adapted from Nymark et al. [2]. Copyright 2008. with permission from Elsevier)

Furthermore, several other interleukins are also released by inflammatory cells [27] upon the phagocytosis of fibers. For example, *IL6* has been shown to be upregulated in airway epithelial cells by NF-κB in response to asbestos exposure [28]. Increased IL6 correlates with increased serum levels of C-reactive protein (CRP), which a follow-up study [29] revealed to be significantly elevated in the serum of asbestos-exposed workers who had developed cancer (lung cancer and mesothelioma) as compared to those who had not developed cancer. In addition, *IL1* and *IL10* have been found to be upregulated by asbestos-induced oxidative stress in vitro [30, 31]. Furthermore, the macrophage Nod-like receptor protein (Nalp3) inflammasome is reportedly activated by asbestos in vitro and has been associated with the pathological increase of IL-1β, in, for example, asbestos-induced mesothelioma. It is well known that IL-1β-driven inflammation promotes the development and invasiveness of several tumor types. Therefore, it has been proposed that Nalp3 inflammasome is an innovative therapeutic target with possible translational significance in asbestos-induced cancer (reviewed in [10, 15, 23].

Apoptosis

Apoptosis plays an important role in the inflammatory process and in the resolution of an inflammatory state. Furthermore, apoptosis protects against the abnormal proliferation of cells with nonrepairable DNA damage (discussed below). Many of the asbestos-induced alterations in the cell should eventually lead to apoptosis. However, the apoptotic pathways seem to be inhibited in asbestos-associated lung carcinogenesis as in many other carcinogenic mechanisms. Low doses of asbestos have been shown to promote S-phase entry and thereby cell proliferation through an EGFR-dependent pathway instead of apoptosis [32]. When apoptosis is bypassed, the asbestos-associated dysfunctions in the mitochondrial respiratory chain maintain the increased release of ROS. Furthermore, the expression and phosphorylation of cAMP-responsive element-binding protein (*CREB*) is thought to be an important regulator of apoptosis in asbestos-induced responses, and silencing of the gene dramatically increases asbestos-induced apoptosis in lung epithelial cells [33]. Similarly, overexpression of the oxidative DNA adduct, 8-OHdG (see section "Biomarkers of Oxidative DNA Damage"), repair enzyme, OGG1, and its translocation to the mitochondria has reduced asbestos-induced apoptosis in HeLa cells [34]. Moreover, gene expression profiling of asbestos-transformed tumorigenic lung cell lines has revealed downregulation of an apoptosis-related putative tumor suppressor *DCC* (deleted in colorectal cancer) [35]. Miura et al. have also produced an apoptosis-resistant T-cell cell line through repeatedly exposing the cells to asbestos. By studying this cell line, they proposed a model mechanism for acquiring resistance to asbestos-induced apoptosis, involving the activation of the genes Src family kinase, *IL-10*, *STAT3*, and *BCL2*. Interestingly, *BCL2* was also found to be significantly upreg-

ulated in the T cells of mesothelioma patients as compared to those of healthy volunteers and asbestosis patients, indicating a role in carcinogenesis [31] (see Chapter 17 for a detailed description). Many other *BCL2*-related genes have been implicated in asbestos-induced apoptotic resistance or carcinogenesis, such as *BNIP3L*, *Bax*, and *Bcl-xl* [36, 37].

MAPK/ERK Pathway

EGFR has proven to be activated by asbestos-induced oxidative stress through phosphorylation [27, 38, 39]. Interestingly, EGFR has also been shown to be overexpressed in malignant mesothelioma, even though no mutations have been detected (see Chapter 17). EGFR, in turn, activates the MAPK/ERK pathway through phosphorylation of ERK1/2 and ERK5 [40], and increased levels of phospho-ERK1/2 and phospho-ERK5 induce proliferation and activation of the AP-1 family members (i.e., the proto-oncogenes c-fos, fra-1, and c-Jun) [41–44]. Low levels of asbestos have been shown to cause cytoplasmic localization of phospho-ERK1/2, and this is followed by AP-1-dependent nuclear localization of cyclin D1 [32]. Cyclin D induces cell cycle reentry through progression from G1 to S phase [45]. Reactivation of the cell cycle in a critical DNA repair stage may lead to a DNA damage bypass allowing cells with oncogenic changes to continue proliferating. Other growth factors such as the insulin-like growth factor (IGF) and platelet-derived growth factor (PDGF) are also known to promote S phase after asbestos exposure [46].

Activation of EGFR also appears to be caused by protein kinase C (PKC)-activated matrix metalloproteinases (MMP) [47], and, for example, MMP2 has been found to be upregulated after combined exposure to chrysotile and cigarette smoke in vivo, which will be discussed in detail below [48]. In accordance, PKCδ and its substrate, adducin, have shown to cause cell proliferation through activation of ERK1/2 in response to asbestos exposure [49, 50]. Noticeably, adducin (ADD1) has also been found to be upregulated in the lung tumors of asbestos-exposed patients when compared to those of non-exposed patients [51]. ERK1/2 appears to be activated by the Src family kinase [52]. Src is a growth-promoting tyrosine kinase, which is activated by the urokinase plasminogen activator (PLAU) pathway involved in tissue reorganization events, such as wound healing. The PLAU pathway appears to be activated by asbestos [53].

Clastogenicity of Asbestos Fibers

In vitro studies have shown that asbestos fibers are clastogenic (able to induce disruptions and breaks in chromosomes), even though they are not mutagenic in the Ames assay [54, 55]. These genetic alterations are thought to contribute to the carcinogenic effects of asbestos. Experimental studies, as well as studies on lymphocytes from asbestos workers, have demonstrated asbestos-induced clastogenicity, involving DNA single- and double-strand breaks, deletions, increased sister chromatid exchanges (SCE), and the formation of micronuclei [55–67]. DNA double-strand breaks are the most severe types of DNA damage and can lead to translocations and chromosomal instability (CIN), since they are more difficult to repair than, for example, DNA single-strand breaks. Crocidolite asbestos appears to be able to induce greater amounts of DNA double-strand breaks than silica and titanium dioxide [64]. In addition, asbestos has been reported to cause abnormal chromosome segregation, which can lead not only to chromosomal deletions and other DNA alterations but also to aneuploidy [58]. The fibers have also been shown to sterically block cytokinesis, leading to binucleated cells and consequently polyploidy [68]. Polyploidy may in turn lead to the chaotic segregation of chromosomes during cell division, thus increasing chromosomal instability (CIN), one of the cornerstones of tumorigenesis (reviewed in [23]).

The chromosomal alterations in lung cancer are very chaotic, and it is difficult to draw any conclusion on whether a specific alteration is associated with asbestos or some other exposure type, for example, tobacco smoke. However, experimental studies show that asbestos exposure is primarily associated with losses and deletions [55, 63, 65–67, 69]. Indeed, most of the asbestos-related chromosomal alterations identified in lung tumor samples to this date are losses (see Chapter 12; [70–75]. In contrast, as mentioned above, polyploidy has also been associated with asbestos exposure and has been identified at high frequency in lung tumor samples from asbestos-exposed patients [74]; see Chapter 12). This is also the case in mesothelioma, which often shows polyploidy of hypodiploid clones (i.e., less than 46 chromosomes; see Chapter 19). Thus, the clastogenic effects of asbestos seem to cause deletions in the genome, while the physical blocking of cytokinesis may induce polyploidy. A good example of these effects is described in one of our studies on the asbestos-associated losses at 2p16. Chromosome 2 is often affected by numerical as well as structural alterations in lung tumors, and we showed that the mean signal count of centromere 2 in lung tumor cells was 2.7 irrespective of the asbestos exposure status of a patient, indicating that the chromosome is often affected by trisomy. In the same study, frequent gains were detected at 2p21 [71]. Another study showed that half of the non-small cell lung cancer (NSCLC) cases examined were affected by gains at 2p21.1-2p14 [76]. Despite this complexity of chromosome 2 alterations in lung tumors in general, we were able to show that a higher frequency of loss of DNA and allelic imbalance (AI) at 2p16 was associated with asbestos exposure [71].

Several experimental settings have shown that asbestos induces micronuclei in lung cells. Micronuclei contain fragments of damaged DNA or even whole chromosomes and are often lost during the subsequent cell divisions, providing an explanation as to why losses and deletions of genomic material are so common following asbestos exposure. Recently, we showed that the mechanism behind the loss of 19p13,

which proven to be more frequent among asbestos-related lung tumors (see Chapter 12) [51], may be the formation of asbestos-induced micronuclei containing specifically 19p13 fragments [75]. In addition, monosomy of chromosome 19 has been detected in vitro in asbestos-transformed human bronchial epithelial cells [77]. Loss of 19p13 has also been identified in mesothelioma [78].

Epigenetic Effects

Epigenetic alterations, such as methylation, are thought to contribute significantly to the development of asbestos-related lung cancer, although the mechanisms behind these alterations are still poorly understood [79–81] (see Chapter 3 for epigenetic mechanisms of carcinogenesis). Nevertheless, *P16/CDKN2A*, which is frequently methylated in lung cancer, has been shown to be significantly more frequently affected by homozygous deletion in asbestos-related lung cancer [70]. The frequencies are comparable to those found in malignant mesothelioma (see Chapters 17 and 19 for detailed discussion on these alterations). Interestingly, one of the gene products of *P16/CDKN2A* ($p14^{ARF}$) positively regulates p53. Thus, alterations in these two genes may be mutually exclusive, explaining why *TP53* mutations are less frequent in mesothelioma, therefore also pointing toward a stronger association between these mutations in lung cancer and tobacco smoking (see Chapter 12 for a more detailed discussion).

The mechanism behind asbestos-induced microRNA regulation is still poorly understood, as in malignant mesothelioma (see Chapter 19), but since differences have been detected in miRNA expression between asbestos-related and non-asbestos-related lung cancer, it is obvious that the exposure is able to also affect these small noncoding genes ([82]; see Chapter 12 for a detailed description). It is, of course, possible that some of them are lost or methylated similarly as described above, for coding genes. However, this needs to be investigated on a deeper level in order to be able to draw any conclusions on these mechanisms.

Finally, the widely versatile process of ubiquitination has been associated with asbestos exposure in lung cancer [83]. The process is involved in various key cellular events, such as DNA repair, cell cycle, and apoptosis, which all seem to be altered in different ways in asbestos-induced lung carcinogenesis. Thus, this pathway is an interesting target for further investigation.

Synergistic Mechanisms Between Asbestos and Tobacco Smoke

Asbestos elevates the risk of nonsmokers contracting lung cancer, but the risk seems to increase in an almost multiplicative manner in smokers, indicating that tobacco smoke and asbestos act as synergistic cocarcinogens [84]. Various joint effects ranging from less than additive to more than multiplicative have been reported, but the generally accepted model that seems to fit the best is a more than additive or less than multiplicative one [85].

Several mechanisms are likely to contribute to the synergistic effects of these two carcinogens. For example, some studies demonstrate that cigarette smoke augments the penetration of asbestos fibers in rat tracheal explants by an oxygen radical-mediated mechanism [86]. Tobacco smoke may also interfere with the clearance of asbestos fibers from the lungs [85]. Furthermore, tobacco carcinogens are known to be adsorbed onto the surface of asbestos fibers, increasing their uptake into the cells [84, 87]. In addition, it has been observed that ROS alter the metabolism of the tobacco carcinogen, benzo[a]pyrene, by inhibiting its detoxification pathways [88]. Yet another hypothesis is that asbestos fibers induce cell proliferation and thereby clonal expansion of cells with heritable tobacco carcinogen-induced alterations in critical genes [89]. As mentioned previously, asbestos is not considered to be able to induce point mutations, although some studies on human lung tumors have linked specific *TP53* mutations, i.e., predominantly exon 9–11 mutations to asbestos exposure [90, 91]. However, we could speculate that at least a part of these mutations would primarily be caused by tobacco-specific carcinogens such as benzo[*a*]pyrene [92]. Indeed, as mentioned above, the frequency of *TP53* mutations is also significantly lower in malignant mesothelioma compared to other cancers, indicating that asbestos fibers are not, or at least not directly, involved in the alterations of this gene (see Chapter 12). It has also been shown that p53 may be phosphorylated at Ser15 following exposure to DNA-damaging agents, including asbestos. Phosphorylation causes stabilization and subsequent transactivation, which consequently leads to sustained expression levels (reviewed in [90]).

Finally, it has been proposed that the synergistic properties of asbestos and tobacco smoke may be caused by separate activation of the ERK genes and JNK1/2, respectively, which both transactivate AP-1, as mentioned above [40]. The cocarcinogenic mechanisms mediated by the transcription factors, nuclear factor-erythroid 2-related factor (NRF2) and aryl hydrocarbon receptor (AHR), which regulate oxidative stress- and tobacco carcinogen-induced gene expression, respectively, are discussed below (see section "Cocarcinogenesis Mechanism of Tobacco Smoking and Inhaled Particulates").

Polycyclic Aromatic Hydrocarbons and Complex Mixtures

Occupational Exposure to Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons arise in the incomplete combustion of fossil and carbonaceous materials and also occur in crude oil deposits. The highest occupational exposures are

Table 10.1 Examples of biomarkers of internal dose, biologically effective dose, and early effects in relation to occupational exposures to PAH and complex mixtures

Examples of exposures	Markers of internal dose	Markers of effective dose	Markers of early biological effects
Involuntary tobacco smoking Coke-oven workers Foundry workers Bitumen workers Petrochemical industry Rubber vulcanizing Diesel exhaust/working in traffic Firefighting Soil remediation Waste handling	Urinary metabolites of tobacco constituents Cotinine (nicotine metabolite) NNAL and NNAL/cotinine ratio 1,3-Butadiene Urinary PAH metabolites 1-Hydroxypyrene and other PAH metabolites	DNA adducts in blood lymphocytes or lung Bulky DNA adducts Anti-B[a]PDE-DNA adducts 8-OxodGuo adducts Protein adducts Hemoglobin adducts Urinary/plasma markers of oxidative DNA damage Excretion of 8-oxo-7,8-dihydroguanine	Cytogenetic aberrations detected in blood lymphocyte culture Micronucleus formation Sister chromatid exchanges Chromosomal aberrations DNA strand brakes in blood lymphocytes (measured by comet assay) Changes in global and gene-specific promoter methylation Shorter telomere length

Abbreviations: *PAH* polycyclic aromatic hydrocarbon, *NNAL* tobacco-specific nitrosamine metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, *anti-B[a]PDE* anti-benzo[a]pyrene diol epoxide, comet assay, alkaline single-cell gel electrophoresis assay

found in petrochemical industry workers, especially in coke-oven workers, and in workers of metal plants and foundries [93]. Sources of indoor PAH exposure include tobacco smoke, meat and fish roasting and frying, and charcoal grilling in poorly ventilated environments [94]. Examples of occupations with PAH exposure are given in Table 10.1. Workers in the petrochemical industry and in foundries are typically exposed to complex mixtures, in which chemical compounds are bound to metal and mineral particulates of respirable size. Some of these metals and minerals are known or suspected lung carcinogens as such; examples include arsenic, some chromium and nickel compounds, cadmium, vanadium, silica, and fibrous minerals including asbestos. PAH levels and the distribution of different PAH compounds between gaseous and particulate phases have been studied in air samples from foundries. While the gas phase contains on average three times more carcinogenic four- and five-ring PAHs, the total PAH load increases with increasing particle size in individual fractions [95–98]. The distribution of PAHs between gaseous and particulate phases is important because the mechanisms and biomarkers of chemical and particle/fiber carcinogenesis are different. While pure PAH procarcinogens are metabolized via the AH receptor-mediated pathway to DNA-reactive intermediates or detoxified and excreted from the body, particulates, some metals, and fibers induce the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and oxidative DNA damage.

Involuntary Tobacco Smoking

Environmental tobacco smoke (ETS) is a significant source of PAH and other tobacco carcinogens for nonsmokers in workplaces, especially in poorly ventilated environments. ETS is a complex mixture of gaseous and particulate-bound compounds, including known carcinogens such as acrolein, aromatic amines, acetaldehydes, benzene, cadmium, 1,3-butadiene, tobacco-specific nitrosamines, and polycyclic aromatic hydrocarbons [98–100]. ETS consists mainly of sidestream smoke emitted from smoldering cigarettes between puffs and to a lesser extent of mainstream smoke exhaled by tobacco smokers [101]. The delivery of different compounds by mainstream and sidestream tobacco smoke is influenced by the efficiency of combustion and differs between tobacco brands due to tobacco blends, ingredients, design, and differences in manufacture. The harmful chemicals in sidestream tobacco smoke are principally responsible for the deleterious health effects of involuntary tobacco smoking. Lodovici et al. studied the PAH content in mainstream and sidestream tobacco smoke from 14 tobacco brands and found that sidestream smoke contained about ten times higher PAH levels than mainstream smoke from most cigarette brands [102]. While the tar content of cigarettes is a good predictor of the release of PAHs in mainstream smoke, PAHs in sidestream smoke do not correlate with tar content [102, 103]. Furthermore, levels of carcinogenic PAH compound benzo[a]pyrene are especially high in sidestream tobacco smoke [102]. Most carcinogenic PAH compounds are present in the particulate phase of tobacco smoke.

Metabolic Activation of PAH Procarcinogens

PAH compounds enter cells as procarcinogens which require metabolic activation to exert their carcinogenic potential. In lung cells, PAH compounds bind to a cytoplasmic aryl hydrocarbon (dioxin) receptor (AHR) which, after ligand binding, is translocated to the nucleus and dissociates from

the cytoplasmic chaperone complex. It then associates with its dimerization partner, ARNT protein, and binds to xenobiotic (dioxin)-responsive elements (XRE) in the promoter (enhancer) regions of AHR-responsive genes, turning on their transcription (e.g., [104, 105]). AH receptor regulates the transcription of several cytochrome P450 (CYP) enzymes, which are involved in the Phase I metabolism of xenobiotics, and also the transcription of a few Phase II enzymes, including UDP-glucuronosyltransferases 1A1 and 1A6, glutathione S-transferase A2, and NAD(P)H:quinone oxidoreductase 1 (NQO1). Generally speaking, Phase I metabolism is responsible for the initial activation step of metabolism, often leading to the formation of reactive intermediates, whereas Phase II metabolism involves the conversion to more polar and water-soluble compounds and detoxification [104, 106].

In the lung, cytochrome P450 enzymes CYP1A1 and CYP1B1 and epoxide hydrolase catalyze the conversion of PAH procarcinogens to proximate carcinogenic metabolites, PAH diols, and CYPs further to ultimate carcinogenic metabolites PAH diol epoxides. CYP1A1, CYP1B1, and a third PAH-metabolizing lung enzyme, CYP2S1, are under the regulative control of the AH receptor. In general, CYP2S1 is a PAH-detoxifying rather than a PAH-activating enzyme. In the presence of an oxidizing agent, such as hydrogen peroxide, CYP2S1 has been shown to oxidize benzo[a]pyrene-7,8-dihydrodiols into epoxides with a high turnover [107]. This finding may have relevance in human exposures to various particulate and complex mixtures that induce oxidative stress.

Reactive metabolites may bind to proteins and DNA, thereby forming adducts, or become detoxified by Phase II enzymes, such as glutathione S-transferases, UDP-glucuronosyltransferases, and sulfotransferases [106, 108]. PAH diols are also metabolized by aldo-keto reductase into reactive PAH *o*-quinones, which are able to form stable and depurinated DNA adducts. Furthermore, PAHs are catalyzed by peroxidase activities into radical cations that form depurinated adducts [106, 109–111].

Bulky DNA adducts, which mainly originate from PAH, are considered a measure of internal dose of PAH and, if not repaired, may lead to DNA damage. Denissenko and colleagues mapped the distribution of benzo[a]pyrene diol epoxide (BPDE)-DNA adducts along exons of the *TP53* gene and observed strong and selective adduct formation at guanine positions in codons 157, 248, and 273. These same codons are the mutational hotspots in human lung cancer [112]. Subsequent studies have shown that methylated CpG dinucleotides are the preferential target for BPDE adduct formation and G:C to T:A transversions at *TP53* codons 157, 248, 249, and 273 [113, 114]. The molecular alterations caused by tobacco-derived PAH and occupational PAH exposures are not separable.

Cocarcinogenesis Mechanism of Tobacco Smoking and Inhaled Particulates

It has long been known in epidemiology that tobacco smoking and asbestos exposure have a synergistic, almost multiplicative effect on lung cancer risk as compared to the risk caused by either exposure alone. The exact mechanisms for the synergism are not known, but the emerging knowledge of the cooperation between the transcription factors and signaling pathways that are induced by tobacco carcinogens and oxidative stress offers a plausible view on cocarcinogenesis. Oxidative stress, together with its effects on cellular structure and function, plays a central role in the carcinogenic process induced by inhaled particulates, including asbestos fibers, silica, and carcinogenic metals, as well as ionizing radiation.

PAH compounds exert their effects via the AH receptor, which regulates the transcription of a number of xenobiotic-metabolizing enzymes by binding to xenobiotic-responsive elements (XRE) in the promoters of responsive genes. Recent research has shown that the AH receptor plays an additional role in the control of cell proliferation and apoptosis, differentiation, and inflammation, for example, via interactions with pRB, EGFR, and NF-κB signaling [104].

Several other transcription factors are linked to AHR, for example, the nuclear factor-erythroid 2-related factor 2 (NFR2), which controls the antioxidant gene battery [115, 116]. NRF2 regulates gene expression via binding to antioxidant responsive elements (ARE) in the regulatory sequences of NRF2-driven genes. Many of the NRF2-regulated genes encode enzymes which are responsible for the detoxification of reactive electrophiles formed by Phase I metabolism by CYPs or for the elimination of reactive oxygen species, including enzymes such as NAD(P)H:quinone oxidoreductase 1 (NQO1), glutathione transferases, UDP-glucuronosyltransferases, aldehyde dehydrogenase, and several antioxidant enzymes [117, 118]. Interestingly, AHR- and NRF2-regulated signaling is coordinated by several mechanisms, for example, *AHR* and *NRF2* genes contain each others' binding elements in their regulatory enhancer regions [115]. Furthermore, induction of the expression of a group of genes, such as detoxification enzyme NQO1, requires both AHR and NRF2 [116].

It has been shown recently that loss of the regulative control of NRF2 in human lung cancer cells may result from several aberrations, such as mutations in the *NRF2* gene or its repressor *KEAP1* [119, 120]. *KEAP1*, which is considered a tumor suppressor, may also be silenced by hypermethylation or the deletion of the chromosomal region 19p [75, 121]. These aberrations, which lead to constant NRF2 activation, may arise as a protective response against reactive eletrophiles and oxygen species or become selected by means of giving a growth advantage and permitting cancer

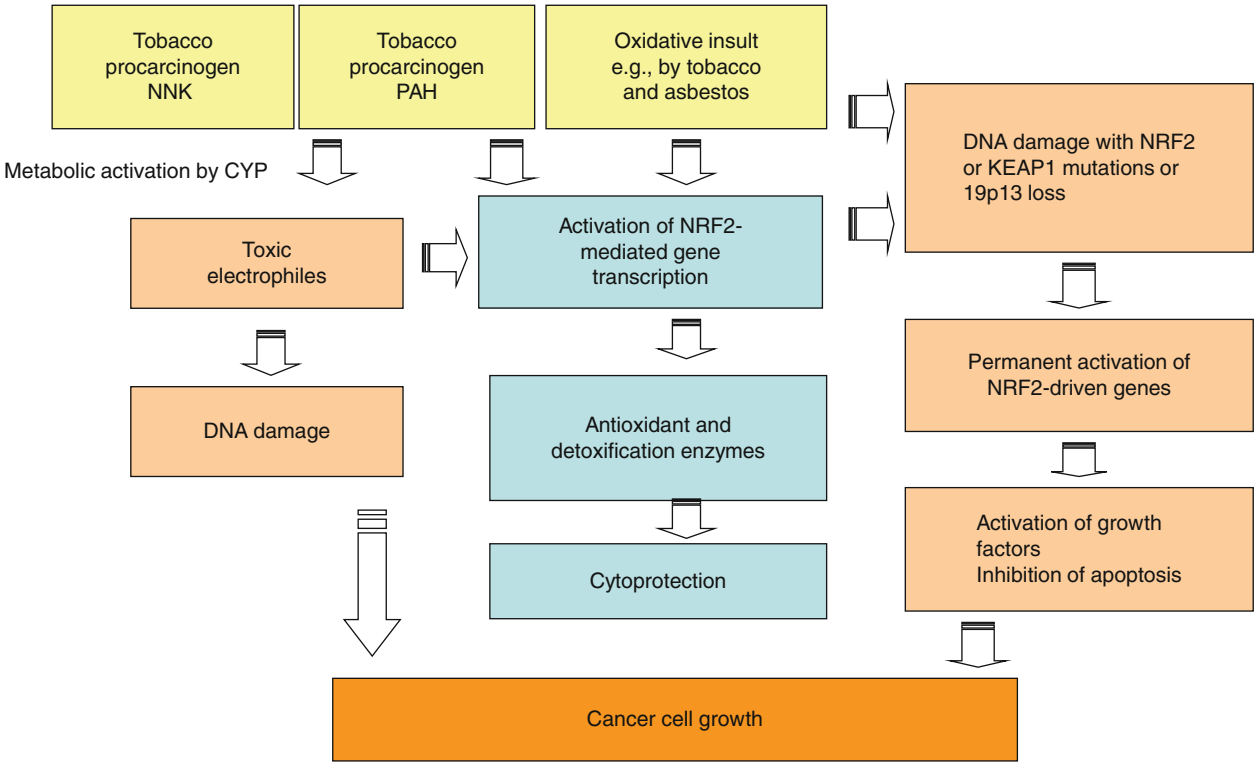

Fig. 10.2 Cocarcinogenesis mechanism of tobacco carcinogens and oxidative stress damage (Reprinted with permission of the American Thoracic Society. Copyright 2013 American Thoracic society. Anttila et al. [108])

cells to avoid apoptosis [108, 122]. Constant NRF2 activation results in overexpression of a number of NRF2-dependent genes, most of them cytoprotective and antioxidant enzymes. Upregulation of NRF2-mediated gene expression seems to involve genes that may promote cancer cell growth, including growth factors such as fibroblast growth factor 13; TGF-α, TGF-β1, and TGF-β2; and growth factor receptors [122]. It has been shown that NRF2 activity regulates the sensitivity of death signals and NRF2 overexpression antagonizes Fas-induced apoptosis [122–124]. Furthermore, one such NRF2-regulated antioxidant enzyme, peroxiredoxin 1 (PRX1), which is commonly upregulated in human cancer, has a dual role as it may provide resistance to oxidative stress in cancer cells by the inhibition of apoptosis-signal regulating kinase 1 (ASK1) activation and subsequent ASK1-induced apoptosis [125]. The enhancement of the oxidative stress and consequent apoptotic pressure by combined exposures to tobacco and asbestos may lead to DNA damage in critical genes, resulting in uncontrolled expression of NRF2-regulated genes, inhibition of apoptosis, and growth advantage to cancer cells. One of these critical aberrations, the deletion of the 19p chromosomal region, is especially common in asbestos-related lung cancer [51, 75]. The postulated mechanism of cocarcinogenesis of tobacco carcinogens and oxidative stress is shown in Fig. 10.2.

Biomarkers

Biomarkers of PAH Exposure

The biomarkers of PAH exposure most commonly used are urinary PAH metabolites, in particular 1-hydroxypyrene. 1-Hydroxypyrene and another urinary biomarker, hydroxybenzanthracene, are noncarcinogenic metabolites and are thought to reflect total PAH exposure. The level of urinary PAH metabolites is influenced not only by occupational exposure but also by diet, tobacco smoking, and environmental air pollution. Typically, in air samples from foundries and petrochemical plants, PAH concentrations are about three orders of magnitude higher than those in environmental exposures. Similarly, urinary 1-hydroxypyrene concentrations reflect the exposure levels well at the group level. PAH-DNA or protein adducts are considered the measure of an effective dose of PAH exposure.

Biomarkers of Oxidative DNA Damage

DNA strand brakes and 8-hydroxyguanine (8-hydroxydeoxyguanosine, 8-OHG) formation are the most commonly used tests for oxidative DNA damage caused by

exposure to PAH and inhaled particulates in the scientific literature. The oxidized DNA product 8-OHG is formed in the reaction of guanine with hydroxyl radical [126]. This mutagenic and carcinogenic DNA product is a good biomarker of oxidative stress and can be determined in urine or circulating white blood cells [126]. 8-OHG levels in urine are also influenced by gender, age, body mass index, and lifestyle factors, such as tobacco smoking, hard physical labor, and diet [127, 128]. DNA strand brakes can be studied by comet assay (alkaline single-cell gel electrophoresis assay) in cultured cells or in the circulating blood lymphocytes of exposed individuals [129]. Tarantini et al. studied the relative contribution of DNA strand brakes and DNA adducts to the diol epoxide metabolite of B[a]P in the cellular effects of pure B[a]P and complex mixtures collected from an urban peri-industrial site and a metallurgical plant [130]. Treatment of HepG2-cultured human hepatocytes with pure B[a]P or with a fraction of atmospheric particles containing soluble PAH did not induce DNA strand brakes in comet assay or the formation of 8-OHG, whereas B[a]PDE adducts were observed with even low concentrations. In contrast, samples filtered from industrial and especially those from urban sites induced DNA strand brakes and the formation of 8-OHG and less BPDE adducts, suggesting that a component other than PAH, possibly particulate matter in the mixture, modulates the genotoxic properties of complex mixtures [130].

The most commonly used biomarkers of internal dose, biologically effective dose, and early effects in relation to occupational exposures to PAH and complex mixtures are listed in Table 10.1.

Metal-Induced Lung Carcinogenesis

Metal-induced carcinogenesis has been covered in detail in several recent reviews [126, 131–142]. For more information regarding metal carcinogenesis, readers are referred to these and other reviews, and for the basic biological mechanisms of carcinogenesis, Chap. 3. The principal mechanisms of metal carcinogenesis are listed in Table 10.2.

Arsenic

Arsenic and its compounds have been identified by IARC as group I human carcinogen, causing cancers of the skin, liver, kidney, bladder, and lung [143]. Globally, arsenite [As(III)] or arsenate [As(V)] is a significant contaminant of drinking water, causing an excess of cancers especially of the skin and

Table 10.2 Mechanisms related to metal-induced lung carcinogenesis

Metal	Target molecule/effect	Result in cellular structure/function
Arsenic	Oxidative stress	Activation of signaling pathways responsive to oxidative stress
	Defective DNA ligation during excision repair	Oxidative damage causing DNA strand breaks and chromosomal aberrations
	Global DNA hypomethylation	Altered gene transcription
	Hypermethylation of promoters of tumor suppressor genes	Loss of function of tumor suppressor genes
Cadmium	Oxidative stress and disruption of cellular antioxidant (glutathione) system	Oxidative damage causing DNA strand breaks and chromosomal aberrations
		Activation of signaling pathways responsive to oxidative stress
	Induction of acquired apoptotic resistance Inhibition of DNA damage repair	Accumulation of inherent DNA damage in proliferating cell population resulting in malignant transformation
	Global DNA hypomethylation	Altered gene expression
	Hypermethylation of promoters of tumor suppressor genes	Loss of function of tumor suppressor genes
Chromium	Oxidative stress via reduction of Cr(VI) to Cr(V), Cr(IV), and Cr(III)	Oxidative DNA damage Activation of signaling pathways responsive to oxidative stress
	Reaction of Cr with DNA	Cr-DNA adducts and DNA-protein and DNA-DNA cross-links
	Dysregulation of mismatch repair	Genomic instability
	Cross-linking complexes in promoters of several genes	Inhibition of gene transcription
	Hypermethylation of promoters of tumor suppressor genes	Loss of function of tumor suppressor genes
Nickel	Weak inducer of oxidative stress	Oxidative DNA damage
	Inhibition of histone acetylation	Inhibition of gene transcription
	Induction of de novo DNA methylation	
	Disruption of iron and zinc homeostasis (zinc finger proteins)	Inhibition of enzymes involved, e.g., in DNA repair, histone methylation, and hypoxic signaling
	Activation of hypoxic signaling	Induction of cell proliferation, migration, invasion, and angiogenesis

bladder. Occupational exposure, via inhalation of arsenic compounds such as arsenic trioxide, arsenic trisulfide, and calcium arsenate, increases lung cancer risk in ore smelters, insecticide manufacture, and sheep dip workers [131].

The inorganic arsenics can be methylated in vivo to form monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in a process of repeated reductions and oxidative methylations, which enhance excretion from the body. However, methylated arsenicals also have a more adverse effect in human cells than the parent compound. MMA and DMA are also ingredients in weed killer chemicals. Trivalent methylated arsenicals are biologically highly reactive and can interact with cellular targets such as proteins and DNA [131, 144–146]. Arsenic metabolism in cells leads to the generation of a variety of reactive oxygen and nitrogen species, including superoxide, singlet oxygen, hydrogen peroxide, the peroxyl radical, nitric oxide, dimethylarsinic peroxyl radicals, and the dimethylarsinic radical [126, 132]. The exact mechanism for the generation of these reactive species is not clear, but the formation of intermediary arsine species or the oxidation of As(III) to As(V) has been suggested [126, 147]. The formation of 8-hydroxyl-2′deoxyguanosine (8-OHdG) DNA adducts is a biomarker of oxidative stress to DNA. Increased levels of 8-OHdG adducts have been detected after exposure to arsenic in cells, in animal models, and in arsenic-induced lesions of human skin [132, 147–149].

Arsenic is not mutagenic in standard assays, but it is genotoxic and induces chromosomal aberrations, sister chromatid exchange, aneuploidy, micronuclei formation, and DNA-protein cross-links [150–153]. Arsenite has been demonstrated by alkaline single-cell gel electrophoresis (comet) assay to induce DNA strand breaks in various human and rodent cells [132, 154–156]. Arsenite-induced DNA strand breaks are caused by ROS production, and breaks may lead to chromosomal rearrangements. Wang et al. [157] have shown that arsenite-induced DNA strand breaks largely result from excision of oxidative DNA adducts and DNA-protein cross-links during excision repair [157]. Arsenic inhibits completion of DNA excision repair via effects on DNA ligase activity perhaps due to being a phosphate analog and interfering with phosphorylation reactions and phosphate transport [132, 158–160].

Arsenic exposure has been related especially with squamous cell histological lung cancer type [161, 162]. Martinez et al. studied gene copy number alterations in squamous cell lung carcinomas from nonsmokers exposed to arsenic in drinking water and observed the most recurrent losses at chromosomal regions 1q21.1, 7p22.3, 9q12, and 19q13.31 and gain at 19q13.33 [163]. These findings are in agreement with the ability of arsenic to induce DNA strand breaks.

Arsenic exposure activates several signal transduction pathways which enhance cell proliferation or reduce antiproliferative signaling, inhibit differentiation, and override the cell cycle checkpoints that control cell division and apoptosis [133].

Epigenetic mechanisms are involved in arsenic-induced carcinogenesis. Arsenic treatment of rat liver cells and human keratinocytes has resulted in reduced expression and activity of DNA methyltransferases, inducing global DNA hypomethylation [134, 164, 165]. Arsenic treatment or exposure has also been associated with the silencing of tumor suppressor genes by hypermethylation of their promoter regions, such as *RASSF1A* and *RPSS3* in human bladder cancer [166], *p16*(*INK4a*) and *RASSF1A* in murine lung cancer [167], *DEPK* in SV-40-immortalized human urothelial cells and in human urothelial (bladder) carcinomas from the arsenic-contaminated area [168, 169], *TP53* in human lung adenocarcinoma A549 cells [170], and *TP53* and *P16*(*INK4A*) in whole blood DNA of people exposed to arsenic in drinking water [171]. Both the global hypomethylation and hypermethylation of promoter regions of tumor suppressor genes are common alterations in malignant tumors. It has also been shown that arsenite changes global histone methylation levels in human lung adenocarcinoma A549 cells [165, 172].

Arsenic is a powerful cocarcinogen and is able to enhance the carcinogenicity of other agents, such as ultraviolet and ionizing radiation, benzo[a]pyrene, N-methyl-N-nitrosourea, diepoxybutane, and methylmethane sulfonate [173–179] in cell and animal models. The interference of arsenic with DNA repair has been suggested as a possible mechanism of cocarcinogenesis. In the study of Chiang and Tsou, which used human lung cell lines, arsenic potentiated the effect of the model PAH procarcinogen, benzo[a]pyrene, to induce BPDE-DNA adducts, without influencing the rate of adduct repair [180].

There is epidemiological evidence of the synergistic effect of ingested arsenic and tobacco smoking on lung cancer risk [181, 182]. A Taiwanese study demonstrated the synergy for the squamous and small cell but not for the adenocarcinoma of the lung [183]. The same group demonstrated that arsenic increased the metabolism of a tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), via activation of CYP2a in mouse liver, and the metabolism of another tobacco carcinogen, benzo[a]pyrene, by enhancement of CYP1A1 expression and activity via the AH receptor with a mechanism involving oxidative stress, in a human adenocarcinoma cell line [184, 185]. CYP enzymes catalyze the initial step (Phase I) in the metabolism of nitrosamine and PAH procarcinogens, which is necessary for the subsequent reactions leading to the formation of DNA-reactive metabolites, as well as detoxification (Fig. 10.2).

Beryllium

Beryllium and beryllium-containing compounds are classified as human carcinogens or likely human carcinogens, causing lung cancer [135, 186]. Much of the human

epidemiological data demonstrating increased lung cancer risk are associated with very high exposures which took place before the 1950s in plants involved in the extraction of beryllium hydroxide from beryl ore, ore refining, and beryllium processing including the production of beryllium oxide, pure beryllium metal, and beryllium copper alloy and the machining of beryllium-containing materials [135].

There is no extensive research data concerning the mechanisms of beryllium-related carcinogenesis. Gordon and Bowser have reviewed the studies on the genotoxicity and carcinogenicity of beryllium [136]. The different chemical forms have had differing effects on mutagenicity and carcinogenicity, and there are no data concerning the beryllium forms relevant to human exposures, i.e., respirable size particles of beryllium metal, alloys, or ceramics [135, 136]. Mammalian test systems have shown evidence of beryllium-induced mutations, chromosomal aberrations, and cell transformation, whereas bacterial tests have been negative [136].

Epigenetic alterations have been detected in beryllium metal-induced rat lung tumors. Belinsky et al. observed hypermethylation of the promoter and loss of transcription in the *p16*(*INK4a*) tumor suppressor gene in 80 % of beryllium-induced rat lung tumors [187].

Cadmium

Cadmium (Cd) is classified as a human lung carcinogen by the International Agency for Research on Cancer [186]. Exposure to Cd is common because the metal is widely used in industry, for example, in electroplating, paints and pigments, welding, and Ni-Cd batteries. Significant amounts of Cd are also released into the environment by human activities [137]. Moreover, Cd is present in the Earth's crust and is selectively taken up by certain edible plants and by, for example, the tobacco plant, making tobacco smoke a significant source of Cd for smokers. The amount of Cd stored in organs depends on their content of a Cd-binding protein, metallothionein. The half-life of Cd in humans is 15–20 years; in lung tissue, Cd is cleared with a half-life of 9 years after quitting smoking [137, 188].

Several mechanisms contribute to the carcinogenicity of Cd (see Table 10.2) [137, 138]. Cd is a weak genotoxic agent and its genotoxicity, i.e., chromosomal aberrations, sister chromatid exchange, DNA strand brakes, and DNA-protein cross-links, is partially mediated by oxygen radical damage [137, 189–191]. Cd is able to induce the generation of ROS in vitro and in vivo, including superoxide anion, hydrogen peroxide, hydroxyl radical, and lipid radicals, in spite of not functioning as a catalyst in the Fenton reaction [138]. It has been proposed that Cd can replace iron and copper in cytoplasmic and membrane proteins, thus increasing the amount of free or chelated copper and iron, which in turn may induce oxidative stress via Fenton reactions [126, 192]. Following exposure to Cd, several transcription factors and pathways are activated that are responsive to oxidative stress, including transcription factors AP-1, NF-κB, and a nuclear factor-erythroid 2-related factor 2 (NRF2) and mitogen-activated protein kinases (MAPKs) signal transduction pathways [138]. MAPKs play an important role in programmed cell death (apoptosis) for the elimination of cells with oxidative DNA damage.

There is evidence that Cd may promote a selective enrichment of cells with genetic damage and resistance to apoptosis, leading to cell proliferation and malignant transformation. The mechanisms of apoptosis resistance induced by Cd are not fully known, but downregulation of several members of the caspase family mediators of apoptosis and reduced expression of the anti-apoptotic gene, *bax*, have been observed in gene expression profiling of Cd-transformed human prostate epithelial cells [193].

The potential of Cd to inhibit the repair of oxidative DNA damage has been demonstrated in several in vitro and in vivo studies, and it is considered a major mechanism of Cd-induced carcinogenesis [137, 194, 195]. Inhibition of DNA damage repair by Cd is thought to be attributable to its effects on enzymes involved in oxidative damage repair, as Cd can be substituted for zinc in zinc finger proteins, resulting in the enzyme's defective repair capacity [137, 196].

The role of epigenetic mechanisms in Cd carcinogenesis is uncertain [134]. In human prostate cells and in another study using rat liver cells, Cd initially induced global DNA hypomethylation followed by hypermethylation after prolonged exposure [197, 198]. In human prostate cells, promoter hypermethylation and reduced expression of *RASSF1A* and *p16* tumor suppressor genes were observed [198]. It is hypothesized that global DNA hypomethylation is associated with Cd-induced cell proliferation [134, 199]. The possible effect of Cd on histone tail posttranslational modifications is not known [134].

Chromium

Chromium VI (hexavalent chromium, CrVI) compounds have been identified as human lung carcinogens [200]. CrVI is widely used in a variety of industries, for example, in paints, metal finishes, stainless steel manufacturing, alloys, welding, and wood treatment. In contrast to other oxidation states of Cr, CrVI is easily transported into cells by an anionic transport system and subsequently reduced to lower oxidation states by a number of reducing agents, such as glutathione, NADPH-dependent glutathione reductase, ascorbate, cystein, lipoic acid, hydrogen peroxide, fructose, and ribose [139, 201]. It is thought that CrIII is unable to cross cell membranes, but recently it has been suggested that certain CrV and CrIII forms generated by reduction in the extracellular space have high permeability through cell membranes [126,

202, 203]. Insoluble Cr compounds can enter cells via phagocytosis. Particulate or water-insoluble CrVI compounds are more potent than soluble species in causing DNA damage, possibly because of the fast clearance of soluble CrVI, whereas poorly soluble particulates may form a persistent source of carcinogenic Cr species in the lung [204, 205].

Intracellular reduction of CrVI is the main source of reactive intermediates and the extensive formation of Cr-DNA adducts and subsequent DNA damage [126, 133, 139]. CrV, when formed, can have a Fenton-like reaction with hydrogen peroxide, generating hydroxyl radical. Other associated reactions can produce thiyl and superoxide radicals [126, 139]. In addition to free radical-induced DNA damage, the formation of Cr-DNA adducts, above other CrIII-mediated DNA cross-links of glutathione, cysteine, histidine, and ascorbate, is responsible for the mutagenicity and genotoxicity of CrVI [126, 140]. Other Cr-induced structural genetic lesions include DNA strand breaks, DNA-protein cross-links, oxidized bases, abasic sites, and DNA inter- and intra-strand cross-links [139, 206].

The DNA damage caused by Cr can lead to dysfunctional DNA replication and transcription and promote genomic instability by dysregulated repair mechanisms, especially by loss of mismatch repair. Microsatellite instability (MSI) reflects the loss of functional mismatch repair mechanism. A Japanese group has compared the presence of replication error phenotype between lung cancers in chromate-exposed and non-exposed individuals. They observed significantly more frequent MSI and repression of DNA mismatch repair proteins hMLH1 and hMLH2 in the lung cancers of chromate-exposed workers [207, 208]. These findings are contradicted by the lung cell experiments by Rodrigues et al., who observed aneuploid phenotype but did not find MSI or reduced expression of mismatch repair proteins in human bronchial epithelial cells malignantly transformed by hexavalent Cr [209]. These differences suggest that replication error phenotype may not be the initial event leading to cancer development in chromate-exposed workers.

In earlier studies on chromate-exposed lung cancer patients, mutations of *RAS* oncogenes and *TP53* tumor suppressor gene were infrequent [210, 211]. However, *TP53* mutations were unusual changes of AT base pairs and double missense mutations [211].

Chromates have induced gene expression changes by epigenetic mechanisms in tumor suppressors and other critical genes both in experimental settings and in vivo. Interesting data have recently been published concerning mechanisms contributing to the cocarcinogenesis of hexavalent Cr and a model polycyclic aromatic hydrocarbon procarcinogen, benzo[a]pyrene. In mouse hepatoma cells, treatment with potassium chromate represses the expression of the benzo[a]pyrene-metabolizing Cyp1a1 enzyme, blocking the detoxification pathway, and consequently enhances the formation of benzo[a]pyrene diol epoxide-DNA adducts [212]. It was shown that Cr cross-links histone deacetylase 1-methyltransferase complexes to the Cyp1a1 promoter and inhibits gene transactivation. The same research group previously demonstrated approximately 50 other benzo[a]pyrene-inducible genes that were repressed by Cr in a similar manner, including receptor-associated kinases, transcription factors, and genes associated with cell cycle regulation, differentiation, and apoptosis [213]. In human lung adenocarcinoma cell line, potassium chromate induced global changes in various histone tail modifications, including an increase in H3K9 dimethylation in the promoter of the DNA mismatch repair gene, *hMLH1*, and a decrease of its expression [214]. Furthermore, hypermethylation of the promoter regions of several tumor suppressor genes, particularly *hMLH1*, *APC*, and *P16* genes, was recently reported in lung carcinomas of patients with over 15 years' occupational exposure to chromates [215, 216].

Nickel

All nickel [Ni(II)] compounds are classified into group I human carcinogens, which can cause nasal and lung cancer, and metallic nickel as possibly carcinogenic to humans (group 2B) [200]. Nickel is an abundant element in the Earth's crust. It is used in the metallurgical industry in the production of stainless steel and alloys, in electroplating, in stainless steel welding, in Ni-Cd batteries, and in the production of nanoparticles [217]. Nickel pollution in the environment originates from the combustion of fossil fuels in vehicles and power plants, industrial sources, waste incinerators, disposal of nickel compounds, and volcanic eruptions. Nickel also deposits in the soil and plants, which increases exposure via food, drinking water, and tobacco smoking.

Inhalation is the main route of exposure for workers exposed to carcinogenic nickel compounds in industry. While both soluble and poorly soluble nickel compounds are considered carcinogenic, water-insoluble compounds, which enter cells via phagocytosis, are readily dissolved in cellular lysosomes and generate high intracellular levels of Ni^{2+} cations and consequently exhibit higher cytotoxicity and genotoxicity [141]. Potential carcinogens are insoluble dusts of nickel subsulfides and nickel oxides, the vapor of nickel carbonyl, and the soluble aerosols of nickel sulfate, nitrate, or chloride [218].

The different mechanisms involved in nickel-induced carcinogenesis have been described in detail in several recent reviews [133, 134, 141, 142]. Although nickel compounds are not mutagenic in traditional mutation tests, they can induce malignant transformation in human and rodent cells [141, 219–223]. Soluble and insoluble nickel compounds induce genetic abnormalities, preferentially in heterochromatin. Genetic aberrations, such as DNA strand breaks, DNA-protein cross-links, deletion/insertion and single gene

mutations, sister chromatid exchanges, micronuclei, and microsatellite mutations, have been observed in mammalian or human cells in vitro [224].

Compared with Cd and Cr, Ni(II) is a weak inducer of oxidative stress [225, 226]. However, the reactivity of Ni(II) with oxygen derivatives can be modulated by chelation with certain histidine- and cysteine-containing ligands, and free radicals may arise from the reaction of Ni(II)-thiol complexes and molecular oxygen or lipid hydroperoxides [226]. G → T transversion mutations, typical of oxidative DNA damage, have been detected in codon 12 of K-ras oncogene in rat renal sarcomas induced with nickel subsulfide and iron [227]. Several nickel compounds have been shown to increase oxidative DNA damage and the formation of 8-hydroxydeoxyguanosine (8-OHdG) adducts in cultured cells and in rat lungs after intratracheal instillation of nickel compounds [228].

Epigenetic mechanisms are considered more important than genetic changes in nickel-induced carcinogenesis (see also Chap. 3 and Fig. 3.2). Nickel binds to heterochromatin rather than euchromatin, where it alters the heterochromatin structure, causing chromatin condensation, inhibition of histone H4 acetylation, and de novo DNA methylation [134, 141, 229, 230]. Histone acetylation is necessary for transcriptional activation. Nickel restricts the acetylation of histone H4 by binding with its N-terminal histidine-18 and by influencing histone acetyltransferase (HAT) activity [142, 231, 232]. Nickel also increases histone H3 lysine 9 dimethylation [233]. Chen et al. demonstrated that nickel inhibits the activation of dioxygenase enzymes, such as histone demethylase MJD1A and DNA repair enzyme ABH2, by replacing the nonheme iron at their catalytic center [234, 235]. The loss of histone acetylation and de novo DNA methylation silences genes, and the silencing of critical genes, such as tumor suppressor genes, contributes to carcinogenesis. The promoter of tumor suppressor gene *p16* has been constantly hypermethylated in the nickel sulfide-induced malignant fibrous histiocytomas of wild-type mice and mice heterozygous for the tumor suppressor *p53* gene [236]. Also, methylation has been observed in the enhancer regions of *RAR-β2*, *RASSF1A*, and *CDKN2A* genes of rat muscle tumors induced by nickel subsulfide [237].

Activation of hypoxic signaling is another main alteration with significance in nickel-induced carcinogenesis. Gene expression profiling with Affymetrix chips on wild-type or the hypoxia-inducible factor-1 (HIF-1) knockout mouse embryo cells found that after $NiCl_2$ treatment, 114 genes were upregulated and 66 genes downregulated in a manner characteristic of the activation of the hypoxic signaling pathway [238]. The HIF-1 transcription factor is a dimer consisting of two subunits, HIF-1α and HIF-1β (ARNT), which is formed in response to low oxygen tension in cells and, together with transcriptional co-activators, regulates the transactivation of HIF-dependent genes. HIF-1α acts as an oxygen sensor, which, in the presence of hypoxia or nickel, avoids ubiquitylation and proteasomal degradation and accumulates in cells [133]. Hypoxic signaling is thought to be one of the pathways that nickel exposure can induce by disrupting cellular iron homeostasis [239, 240]. In hypoxic cancer and stromal cells, HIF-1 transactivates growth and survival factors, such as VEGF, FGF, PAI-I, adrenomedullin, and NOS, which induce endothelial cell proliferation, migration, invasion, and angiogenesis [141].

Nickel influences carcinogenesis through a number of mechanisms not described in detail here, such as by inhibiting DNA repair, inducing *TP53* mutations, and influencing c-Myc, NF-κB, and MAPK signaling pathways, among others. Nucleotide and base excision repair pathways are impaired by nickel compounds, at least partially by the damage of zinc fingers in DNA repair proteins [241]. Nickel compounds induce carcinogenesis by a number of different mechanisms, including genetic and epigenetic changes, affecting signal transduction pathways, especially hypoxic signaling, and inhibiting DNA repair. There is evidence that nickel interferes with cellular metabolism by disrupting iron homeostasis and inhibiting the function of iron-dependent enzymes.

Mechanisms of Ionizing Radiation-Induced Carcinogenesis

Exposure via inhalation to uranium-containing particles and radon decay products, including high linear energy transfer (LET) alpha-particles, through the mining and processing of ore for nuclear power and weapons is associated with increased lung cancer risk [242]. Uranium is a radioactive heavy metal, the radioactivity of which is attributable to the ^{222}Rn and ^{220}Rn isotopes and their decay products. Studies among miners have been complicated by complex exposures to particulate and non-particulate matter in mines, including arsenic, silica, and diesel exhaust [243, 244].

Ionizing radiation (IR) produces reactive oxygen and nitrogen species that are responsible for oxidative stress and inflammatory response. The inflammatory reaction and oxidative damage are dependent on the dose of IR. Large deletions resulting in partial or complete deletion of entire genes and loss of heterozygosity in the neighboring chromosomal regions are the predominant event induced by alpha-irradiation in vitro [244, 245]. High-LET alpha-emitters including radon, plutonium, and Thorotrast induce double-strand breaks and clustered lesions, which are more difficult to repair than single-strand breaks and depurinated, oxidized, or deaminated bases, produced by low-LET X-rays and gamma-rays [246–250]. High-LET alpha-emitters also induce genomic instability through the inactivation of DNA mismatch repair [251, 252]. Most DNA damage produced by IR is repaired by base excision repair, and nucleotide excision repair, double-strand break repair, and mismatch repair

have lesser roles [253]. Erroneous rejoining of double-strand breaks can result in genomic instability.

In normal cells, IR induces apoptosis or cellular senescence through increased expression of tumor suppressor genes *P16*(*INK4A*) and *TP53* via the DNA damage response. An early study has reported a predominance of the *TP53* codon 249 AGGarg → ATGmet mutation in lung cancer of uranium miners, whereas subsequent studies have failed to show any mutational hotspots related to radon exposure [254, 255]. There is evidence that epigenetic changes are related to exposure to IR and its early biological effects. The cumulative exposure to radon gas in Chinese uranium miners correlated positively with promoter hypermethylation of the *P16*(*INK4A*) tumor suppressor and *O*^{6}*-methylguanine-DNA methyltransferase* (MGMT) DNA repair genes in sputum [256]. In another cohort of New Mexico uranium miners, exposure to radon gas did not increase the aberrant methylation of these genes in sputum, as compared to exposure to tobacco smoke alone [257]. Belinsky et al. have shown a higher prevalence of *P16*(*INK4A*) promoter methylation in the lung adenocarcinomas of workers exposed to ^{239}plutonium than that among non-exposed controls [258].

Conclusion

Many carcinogenic chemicals, including polycyclic aromatic hydrocarbons, present in combustion products and tobacco smoke, enter cells as procarcinogens and require metabolic activation by cytochrome P450 (CYP) enzymes to exert their deleterious effects, including binding to DNA and formation of DNA adducts which, if not repaired, may lead to mutations in critical genes and cancer initiation. The induction of oxygen radical damage is considered the main mechanism of particle and fiber carcinogenesis. In addition, asbestos fibers are clastogenic, giving rise to chromosomal aberrations. Carcinogenic metals are thought to induce oxidative stress-mediated DNA damage. Recent studies have shown that carcinogenic metals may replace metal ions, such as iron and zinc, in critical enzymes involved in DNA repair, histone methylation, and hypoxic signaling, for example. Epigenetic carcinogenic mechanisms have recently been found to play a larger role than previously thought, in environmental carcinogenesis.

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