PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 5.

Tobacco smoke and its constituents

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This chapter addresses the concordance in studies of carcinogenicity and mechanisms between experimental animals and humans for tobacco smoke and its constituents. Volume 100E of the *IARC Monographs* updated the literature on tobacco smoke and the evaluations of its carcinogenicity (IARC, 2012b). It concluded that there was *sufficient evidence* that tobacco smoking causes multiple types of cancer in humans, including (to varying extents) cancers of the lung, oral cavity, pharynx, oesophagus, stomach, colorectum, liver, pancreas, nasal cavity and paranasal sinuses, larynx, uterine cervix, ovary (mucinous), urinary bladder, kidney, ureter, and bone marrow (myeloid leukaemia).

Coherence: carcinogenicity of tobacco smoke in humans versus experimental animals

Volume 100E and previous *IARC Monographs* and certain reviews on tobacco smoke have summarized the literature on animal studies of the carcinogenicity of tobacco smoke (IARC, 1986, 2004, 2012b; Hecht, 2005). These studies demonstrated that cigarette smoke can induce tumours of the lung and nasal cavity in mice and rats and tumours of the larynx in hamsters. Some recent studies not included in the evaluations in Volume 100E have consistently established the carcinogenicity of cigarette smoke to the lung in the A/J mouse, where it produces adenoma and adenocarcinoma, as well as causing emphysema (Stinn et al., 2010, 2013). The A/J mouse, which is highly susceptible to lung tumour development, appears to present a relatively reproducible system for the induction of lung tumours, both benign and malignant, by cigarette smoke.

Although these studies have established animal models for the study of tobacco smoke carcinogenesis and, in aggregate, support the epidemiological findings that smoking is a cause of cancers of the lung and larynx in humans, specific problems associated with animal studies of tobacco smoke exposure have been recognized. Many of these issues result from the fact that most laboratory animals are obligate nose breathers and, thus, do not inhale tobacco smoke *voluntarily and habitually* in the same way in which humans smoke tobacco products (Wynder and Hoffman, 1967). Constant whole-body exposure of rodents to cigarette smoke, often at relatively high concentrations, produces avoidance reactions, stress, weight loss, and other indicators of toxicity that are widely different from

the human responses to voluntary inhalation driven by the desire for recurring small doses of nicotine.

There are some mechanistic differences as well. For example, mutations in the *KRAS* gene are frequently observed in lung adenocarcinoma in humans, as are K-*ras* mutations in lung adenocarcinoma in mice; however, the mutation frequency is not increased and the mutation spectrum is not altered in mice exposed to cigarette smoke (Hutt et al., 2005; DHHS, 2010; Stinn et al., 2013). Taken together, there is only moderate concordance between the carcinogenic and mechanistic effects of tobacco smoke evident in laboratory animals and epidemiological observations in humans (Witschi, 2007).

Concordance: carcinogenicity of tobacco smoke in humans versus carcinogenicity of tobacco smoke constituents in experimental animals

There is considerable concordance between the known carcinogenic properties of many tobacco smoke constituents and the multiple target tissues of tobacco smoke as demonstrated in epidemiological studies (IARC, 2012b).

It should be noted that carcinogenicity assays of pure compounds generally do not suffer from the above-mentioned operational difficulties with respect to tobacco smoke (Witschi, 2007).

With respect to lung cancer, multiple polycyclic aromatic hydrocarbons (PAHs) and the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are found in the smoke of all cigarettes tested to date (IARC, 2004). There is abundant evidence attesting to the ability of PAHs to induce lung cancer in laboratory animals (IARC, 2010). Similarly, NNK is a powerful lung carcinogen, inducing adenocarcinoma of the lung in rats, mice, and hamsters independent of the route of administration and frequently at very low doses (Hecht, 1998). Thus, PAHs and NNK are widely considered to be causes of lung cancer in smokers. PAHs – and their diol epoxide metabolites in particular – produce mutations in *TP53* and *KRAS* that are similar to those observed in lung tumours from smokers (DHHS, 2010). In recent nested case–control studies within prospective cohorts, biomarkers of PAH and NNK exposure were associated with risk of lung cancer, after correction for duration and intensity of smoking (Hecht et al., 2013).

1,3-Butadiene is another compound likely to be involved in the etiology of lung cancer in smokers. It is found in relatively high concentrations in tobacco smoke and is a powerful lung carcinogen in mice, but not in rats (IARC, 2008).

Other tobacco smoke compounds with the lung as a target tissue/organ in some animal studies include isoprene, ethylene oxide, ethyl carbamate, benzene, and various metals (Hecht, 2011).

The oral cavity, pharynx, and oesophagus of rats are established target tissues of the tobacco-specific nitrosamine *N*′-nitrosonornicotine (NNN), and in particular its (*S*) enantiomer (Hecht, 1998; Balbo et al., 2013). NNN is found in the smoke of all tobacco products (IARC, 2007). *N*-nitrosodiethylamine is another tobacco smoke constituent that induces oesophageal tumours in rats, although its levels in smoke are considerably lower than those of NNN. One nested case–control study found a strong relationship between levels of NNN and its glucuronides in urine, collected years before diagnosis, and oesophageal cancer, but not lung cancer, in smokers, after correction for duration and intensity of smoking (Yuan et al., 2011). This indicates considerable concordance between target tissues of NNN and NNK in rats and observations of cancer incidence in smokers (Stepanov et al., 2014).

Carcinogenicity studies in laboratory animals and studies in humans exposed occupationally have established aromatic amines such as 4-aminobiphenyl and 2-naphthylamine as human bladder carcinogens (IARC, 1987, 2012a). These and other aromatic amines are components of mainstream cigarette smoke (Xie et al., 2013). There is also considerable mechanistic evidence from studies of haemoglobin adducts consistent with the proposal that 4-aminobiphenyl is responsible for bladder cancer in smokers (Castelao et al., 2001; IARC, 2012a).

Benzene is a leukaemogen in humans, and it occurs in considerable quantities in cigarette smoke (IARC, 1987, 2012a). The uptake of benzene by smokers has been demonstrated conclusively by biomarker studies (Hecht et al., 2010). Thus, it is likely that benzene is responsible for leukaemia in smokers, although it does not cause leukaemia in rodents (IARC, 2012a).

Multiple tobacco smoke carcinogens have produced tumours of the upper respiratory tract. Tumours of the larynx, nose, and trachea as well as of the pharynx and oesophagus were induced in inhalation studies with benzo[*a*]pyrene, an archetypal PAH, in hamsters (IARC, 2010). Tumours of the nose have also been observed in rats treated with tobacco-specific nitrosamines (Hecht, 1998; Balbo et al., 2013). Inhalation

studies with formaldehyde and acetaldehyde produced nasal tumours (IARC, 1985, 2006, 2012a).

Tobacco smoke contains compounds that are carcinogenic to the colorectum in rats, most notably certain heterocyclic aromatic amines (IARC, 2004, 2012b; Hecht, 2012). With respect to induction of liver cancer by tobacco smoke, there is coherence with furan and *N*-nitrosodimethylamine, which are liver carcinogens in rats (Peto et al., 1991; NTP, 1993), whereas NNK and its major metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) induce pancreatic cancer in rats (Hecht, 1998).

Collectively, there is considerable concordance between established sites of tobacco smoke carcinogenicity in humans and target tissues in experimental animals of individual carcinogens present in tobacco smoke.

Concordance: overall mechanism of cancer induction in humans versus laboratory studies

Fig. 5.1 presents a widely accepted mechanistic framework describing the events that occur in smokers and lead to the eventual development of lung cancer (IARC, 2004; DHHS, 2010, 2014). This scheme is for lung cancer because it is for this disease that the most data are available.

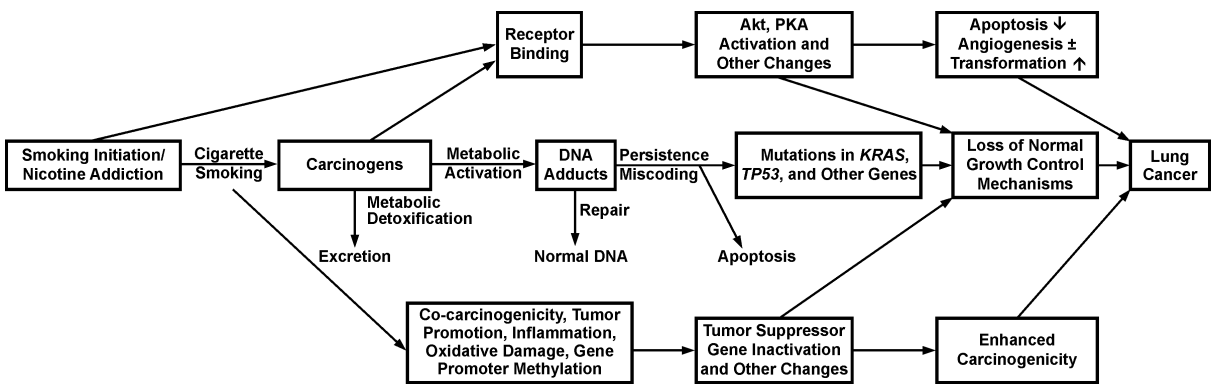
The central pathway of Fig. 5.1 in particular shows great coherence with established genotoxic mechanisms by which many carcinogens, including most of the more than 70 established carcinogens in cigarette smoke, drive the process of cancer induction. Thus, exhaustive mechanistic studies carried out both in vitro and in laboratory animals since the middle of the 20th century and continuing today provide solid evidence that most carcinogens, either directly or after metabolism catalysed by multiple cytochrome P450 enzymes, react with nucleophilic sites in DNA to form covalent binding products called DNA adducts.

There are cellular repair systems that have evolved to repair these DNA adducts and restore the normal structure of DNA. These repair systems are crucial because certain rare DNA repair-deficiency syndromes, such as xeroderma pigmentosum, lead to a high susceptibility to cancer development. Thus, DNA adducts, if left unrepaired, can persist and cause DNA replication errors that may lead to mutations. If these mutations occur in important regions of critical growth control genes, such as the oncogene *KRAS* or the tumour suppressor gene *TP53*, cellular growth processes become severely dysregulated, resulting in uncontrolled cell proliferation and cancer.

There are convincing data from studies of smokers and lung cancer patients that illustrate coherence of these observations in humans with the results observed in the plethora of mechanistic studies noted above. Carcinogen uptake by smokers has been unequivocally demonstrated by biomarker studies that compare levels of carcinogens and their metabolites in the urine of smokers and non-smokers (Hecht et al., 2010). These studies leave no doubt that exposure to multiple carcinogens, including tobacco-specific nitrosamines, PAHs, aromatic amines, and various volatile compounds including benzene and 1,3-butadiene, is significantly higher for smokers than for non-smokers.

These and related studies also show that virtually all of these carcinogens are metabolized by cytochrome P450 enzymes, resulting

Fig. 5.1. Mechanistic framework describing events involved in lung carcinogenesis in smokers. Adapted with permission from DHHS (2010).



in the formation of highly reactive metabolites that react with DNA to produce adducts. The induction of the cytochrome P450 1A1 enzyme in the lungs of smokers via activation of the aryl hydrocarbon receptor, resulting in the conversion of benzo[*a*]pyrene and related compounds to their DNA-reactive forms, is a frequently observed and consistent finding in the literature on the effects of cigarette smoking (DHHS, 2010).

Many studies have demonstrated the presence of multiple DNA adducts in the lungs of smokers, generally at higher levels than those in non-smokers. Although there is still room for further elaboration of the specific DNA adducts involved in this process, there can be little doubt about the higher levels of DNA damage in the lung tissue of smokers compared with non-smokers (IARC, 2004; Phillips and Venitt, 2012).

Consistent with these data are the common findings of mutagenicity in urine of smokers and sister chromatid exchange in peripheral lymphocytes of smokers (IARC, 2004).

Multiple recent studies with currently available DNA sequencing methods have demonstrated that DNA adducts in the lungs of smokers result in mutations.

Greenman et al. (2007) studied mutations in the coding exons of multiple protein kinase genes in lung cancer and other cancers. Lung cancers were among those with the most somatic mutations (4.21 per megabase); the authors attributed this to frequent exposure to exogenous mutagens (Greenman et al., 2007).

In another study, 188 primary lung adenocarcinomas were sequenced. Analysis of 247 megabases of tumour DNA sequence identified 1013 non-synonymous somatic mutations in 163 of the 188 tumours, including 915 point mutations, 12 dinucleotide mutations, 29 insertions, and 57 deletions. The analysis identified 26 genes mutated at significantly elevated frequencies, including *TP53*, *KRAS*, *CDKN2A*, and *STK11*, consistent with other studies and with the known involvement of these genes in growth control. Mutations were found most frequently in *TP53* and *KRAS* (Ding et al., 2008).

Another study examined a small cell lung cancer cell line. More than 22 000 somatic substitutions were identified, among which were 134 in coding exons. G → T transversions were the most common (34%), followed by G → A transitions (21%) and A → G transitions (19%), similar to earlier data in many studies (Pleasance et al., 2010).

Another investigation focused on a non-small cell lung cancer from a patient aged 51 years who had smoked 25 cigarettes per day for 15 years before excision of the tumour, which was histologically characterized as an adenocarcinoma. Single nucleotide variants were common, mostly at GC base pairs, frequently G → T transversions. Approximately 17.7 mutations per megabase were observed, for a total of more than 50 000 single nucleotide variants. At least eight genes in the *EGFR-RAS-RAF-MEK-ERK* pathway were either mutated or amplified (Lee et al., 2010).

These results are fully consistent with those reported earlier (DHHS, 2010) and with data in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (http://www.sanger.ac.uk/genetics/CGP/cosmic/) and the IARC TP53 database (http://www-p53.iarc.fr/), which store and display somatic mutations in *TP53* and *KRAS* as well as other genes important in cancer. Overall, these results are coherent with the induction of multiple mutations in critical growth control genes by metabolically activated carcinogens in cigarette smoke, although it should be recognized that other processes downstream of carcinogen exposure probably also contribute to the mutation load.

In aggregate, these studies present a coherent mechanism based on multiple studies, including chemical analyses, measurements of mutation induction, and tests in laboratory animals as well as biochemical and molecular biological evaluations of human tissues, blood, and urine. The data are consistent and convincing with respect to the central track of Fig. 5.1.

It is clear that other processes are involved. Certain compounds in tobacco smoke, or their metabolites, may interact directly with cellular receptors. This can lead to activation of protein kinases, growth receptors, and other molecules that can contribute to carcinogenesis (Chen et al., 2011). It is well established that tobacco smoke contains inflammatory substances, resulting in enhanced pneumocyte proliferation, activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and tumour promotion (Takahashi et al., 2010). There are also co-carcinogens that undoubtedly contribute to the overall mechanism of tobacco smoke carcinogenesis. Furthermore, cigarette smoke induces oxidative damage, altered gene promoter methylation, dysregulation of gene expression by microRNAs, and chronic cell injury and cytotoxicity with regenerative proliferation as an amplifying factor, all of which can

contribute to the overall carcinogenic effect (IARC, 2012b; Milara and Cortijo, 2012; Momi et al., 2014).

In summary, cigarette smoking represents a potent combination of biological effects associated with carcinogenesis, coherent with landmark publications dating back more than 60 years (Hecht and Szabo, 2014).

Disclaimer

We thank J.A. Ross and C.E. Wood for their helpful comments on this manuscript. This article was reviewed by the National Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency and approved for publication. Approval does not signify that the contents reflect the views of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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