Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Toxicology

Role of epigenetics in tumor induction by non-genotoxic carcinogens

Volodymyr P. Tryndyak

Abstract

Non-genotoxic carcinogens are chemicals that cause the development of tumors through the indirect induction of neoplastic transformation without altering the DNA sequence. The mechanisms of non-genotoxic carcinogenesis are very complex and there is no common mode-of-action that characterizes all non-genotoxic carcinogens making detection of these carcinogens difficult. Recently, epigenetic alterations have been suggested as key cancer-related events induced by non-genotoxic carcinogens. The goal of this minireview is to summarize current knowledge on the similarities and differences in epigenetic changes induced by known non-genotoxic carcinogens, such as phenobarbital, WY-14,643, arsenic, methapyrilene, nickel and 2,3,7,8-tetrachlorodibenzo-p-dioxin, and estimate a potential of using these findings to establish assays for carcinogens detection with non-genotoxic characteristics. This minireview highlights alterations in DNA methylation, histone modifications, and changes in microRNAs as the promising epigenetic mechanisms with respect to carcinogenic potential assessment of non-genotoxic chemicals. The understanding of epigenetic mechanisms during carcinogenesis induced by non-genotoxic carcinogens may help identify new biomarkers for the evaluation of carcinogenic potential of non-genotoxic carcinogens and may contribute to human health risk assessment.

Addresses

Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR 72079, USA

Corresponding author: Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. Food and Drug Administration, 3900 NCTR Road, Jefferson, AR 72079, USA. (volodymyr.tryndyak@fda.hhs.gov)

Current Opinion in Toxicology 2017, **6**:42–49

This review comes from a themed issue on **Genomic Toxicology: Epigenetics**

Available online 31 August 2017

For a complete overview see the Issue and the Editorial

http://dx.doi.org/10.1016/j.cotox.2017.08.004

2468-2020/Published by Elsevier B.V.

Keywords

Non-genotoxic, Carcinogens, Epigenetics, Carcinogenesis, DNA methylation, Histone modifications, microRNA.

1. Introduction

Carcinogenesis is a multistage process that consists of initiation, promotion, and progression stages. Chemicals or environmental factors may act at any of these stages to induce and/or enhance the carcinogenic process. Based on their mode-of-action, carcinogens can be classified as genotoxic or non-genotoxic [1]. The main characteristic of genotoxic carcinogens is their ability to interact directly with DNA to form covalent adducts that result in mutations and genetic aberrations. Non-genotoxic carcinogens can induce carcinogenesis without direct interaction with DNA and do not share a common carcinogenic mechanism that is associated with each chemical [2]. Non-genotoxic carcinogens are characterized by a variety of carcinogenic mechanisms, including the induction of oxidative stress, xenobiotic receptor activation, peroxisome proliferation, receptor and non-receptor mediated endocrine modulation, regenerative cell proliferation, the induction of inflammatory responses, and the evasion of apoptosis [2]. Non-genotoxic carcinogens are negative in *in vivo* and *in vitro* mutagenicity tests, which makes their detection, evaluation, and risk assessment difficult. The absence of DNA alterations during non-genotoxic carcinogen-induced carcinogenesis is forcing the scientific community to look for new markers that can be used for the evaluation of the carcinogenic potential of non-genotoxic carcinogens.

Accumulated evidence over the past decade has indicated the importance of epigenetic alterations in carcinogenesis [3]. Epigenetics is defined as heritable modifications in gene expression that do not involve a change in the DNA sequence, with characteristics that overlap with the non-DNA interacting properties of non-genotoxic carcinogens. Changes in the epigenetic profile of normal cells, due to exposure to drugs and toxicants, especially those that exert a non-genotoxic mode-of-action, may alter gene expression patterns, leading to neoplastic cellular transformation. Furthermore, these epigenetic perturbations may create an environment susceptible to cancer-prone mutations in the cells after exposure to non-genotoxic carcinogens through mutational aberration of gene activity via mainly deamination of a methylated cytosine and induction of genomic instability at repeat-rich DNA regions. Currently, changes in DNA methylation, histone modifications, and alterations in microRNA (miRNA) expression are the main epigenetic events during carcinogenesis induced by chemicals and other

environmental agents. The goal of this review is to summarize current knowledge on the similarities and differences in epigenetic changes induced by known non-genotoxic carcinogens, such as phenobarbital, WY-14,643, arsenic, methapyrilene, nickel and 2,3,7,8-tetrachlorodibenzo-p-dioxin, and estimate a potential of using these findings to establish assays for carcinogens detection with non-genotoxic characteristics. Relevant examples of alterations in DNA methylation, histone modifications, and changes in microRNAs as the most promising epigenetic mechanism with respect to carcinogenic potential assessment non-genotoxic carcinogens will be discussed.

2. Alterations of DNA methylation during carcinogenesis induced by non-genotoxic carcinogens

Global DNA hypomethylation and gene-specific hypermethylation are well-known primary epigenetic regulators of chromatin organization and gene expression. The induction and promotion of tumor development are associated with global DNA hypomethylation, which may increase the frequency of genomic mutation [4], induce the expression of oncogenes [5], lead to the loss of genomic imprinting [6], and cause the reactivation of repetitive sequences. During tumor development, extensive hypomethylation of DNA occurs in DNA repetitive elements and inter-genic regions. This may facilitate chromosomal abnormalities and genomic instability [7]. In addition, alteration of gene-specific methylation can lead to changes of gene expression and malignant cellular transformation [9]. Carcinogens may induce gene-specific promoter hypermethylation leading to the silencing of tumor suppressor genes, while the activation of oncogenes, which were silenced in normal tissues, may be caused by hypomethylation of the regulatory regions, therefore these epigenetic changes may contribute to development of cancer [9]. In order to understand and define specific epigenetic markers, including DNA methylation, that would help us identify non-genotoxic chemicals with carcinogenic potential it is important to summarize our knowledge on perturbations in DNA methylation pattern after exposure to known non-genotoxic carcinogens using animal and *in vitro* models.

Phenobarbital was one of the first therapeutic drugs to be investigated for its cancer-inducing potential with non-genotoxic mode-of-action. Phenobarbital induces global and CG-rich region-specific hypomethylation in the livers of the tumor-prone B6C3F1 mice [10]. Importantly, changes in DNA methylation are more pronounced in the livers of tumor-prone B6C3F1 mice as compared with the tumor-resistant C57BL/6 mice and susceptibility to phenobarbital-induced carcinogenesis inversely associated with capacity to maintain normal pattern of DNA methylation in mice livers [10]. Treatment of primary hepatocytes obtained from the livers of tumor-prone B6C3F1 mice with phenobarbital and another non-genotoxic liver carcinogen, diethanolamine, induced a similar hypomethylation pattern in the CG-rich regions of DNA [11]. These results provide evidence for an epigenetic mechanism being involved in phenobarbital-induced mouse liver tumorigenesis. Furthermore, several studies have suggested that alterations in the DNA methylation pattern of cancer-related genes, such as gene-specific hypomethylation of chymase 1 (*Cma1*), tyrosine kinase non receptor 2 (*Tnk2*), transcription factor 4 (*Tcf4*), transforming growth factor beta receptor 2 (*Tgfbr2*), protein tyrosine phosphatase receptor type O (*Ptpro*), ral guanine nucleotide dissociation stimulator (*Ralgds*), and an increase in the promoter methylation of cyclin dependent kinase inhibitor 2A ($p16^{INK4A}$), may also play a causative role during early stages of tumorigenesis induced by phenobarbital [8,12].

The importance of DNA hypomethylation as a promoting factor for the clonal expansion of initiated cells has also been shown with respect to one of the most extensively studied classes of liver non-genotoxic carcinogens - peroxisome proliferators [13]. Treatment of mice with the a peroxisome proliferator activator receptor-α agonist WY-14,643, a potent tumor-promoting agent in rodent liver, resulted in a rapid decrease in global DNA methylation as well as region-specific changes in DNA methylation [14]. In addition, short-term exposure of B6C3F1 mice to WY-14,643 induces hypomethylation of *c-Myc* proto-oncogene that was associated with an enhanced cell proliferation in the mice livers [15]. These data clearly demonstrate that global and gene-specific DNA methylation potentially may play a causative role in liver carcinogenesis induced by non-genotoxic carcinogen WY-14,643.

Alterations in DNA methylation is an important epigenetic mechanism of arsenic carcinogenicity, a well-known human carcinogen with weak mutagenic properties that exerts multiple non-genotoxic mechanisms of action [16]. Several studies have demonstrated that hypomethylation of DNA is associated with malignant transformation and occurs at early stages of arsenic-induced hepatocarcinogenesis. Thus, malignant *in vitro* transformation of the rat liver epithelial cell line TRL 1215 exposed to arsenic was accompanied by global DNA hypomethylation [17]. The extent of DNA hypomethylation was positively correlated with the tumorigenicity of the cells upon inoculation into nude mice, which suggested a causative role of DNA hypomethylation in arsenic-induced malignancy [18]. Additionally, gene-specific DNA methylation was associated with arsenic exposure in urothelial cells originating from human urinary bladder epithelium, a primary target tissue in arsenic-induced cancer [19]. The levels of inorganic arsenic and its metabolites in the exfoliated urothelial cells were associated with the altered