Review

Epigenetic Mechanisms of the Carcinogenic Effects of Xenobiotics and *in Vitro* Methods of Their Detection

(carcinogenesis / nongenotoxic / cell communication / signal transduction / cytokinetics / xenobiotics / *in vitro* detection)

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Abstract. Carcinogenesis is associated with various epigenetic mechanisms, which can alter intra- and intercellular communication and gene expression and thus affect cytokinetics, i.e. regulation of cell proliferation, differentiation, and apoptosis. These processes lead to a loss of homeostatic control. In addition to "classical" epigenetic events such as DNA methylation and histone acetylation, the major mechanisms include changes in concentrations of signal molecules (hormones, growth factors, fatty acids, etc.), modulation of cell receptors and drug-, hormone- and fatty acid-metabolizing enzymes, oxidative stress, and interference with intracellular signal transduction pathways. Multidisciplinary and multibiomarker approach is necessary for setting up a battery of specific biochemical, molecular, and cellular *in vitro* methods detecting the epigenetic carcinogenic potential of individual chemicals or their environmental mixtures. This approach is based on studies of modes of action of xenobiotics at various levels, including the molecular mechanisms and modulations of cytokinetics, each of them having its specific predictive value.

Carcinogenesis is a multistep process involving complex interactions of mutagenic (genotoxic) and epigenetic (nongenotoxic) events (Pitot and Dragan, 1991). Any mutagenic event irreversibly alters the genomic information of the cell. By definition, "an epigenetic event can be either a stable or reversible alteration in the expression of the genomic information at the transcriptional (turning on and off a gene at the DNA level), translational (altering the stability of the genetic message at the RNA level) or postranscriptional (i.e. phosphorylation of a protein) levels" (Trosko et al., 1998). However, understanding the difference between mutagenic and epigenetic events represents a complicated task. A mutated (initiated) cell is maintained in a "latent" stage by integrated regulation systems. Various groups of endogenous or exogenous compounds operating through the epigenetic mechanisms have the potential to promote clonal expansion of such a cell (Purchase, 1994; Hui and Makuuchi, 1999). For this type of activity, the expression "epigenetic toxicity" has been suggested in the recent years. Epigenetic toxicology is considered as a new paradigm to provide a unifying mechanism for diverse toxicant-induced biological endpoints (Trosko et al., 1998).

Compounds with epigenetic carcinogenic activities have apparently multiple effects on target cells, depending on the compound involved (Klaunig et al., 1998). The most significant consequences include alteration of cell proliferation, differentiation, or apoptosis. In addition to "classical" epigenetic events such as DNA methylation and histone acetylation, many other mechanisms may underlie changes in gene expression and disturbances of cytokinetic processes: structural and functional alterations of cytoplasmic membranes, inhibition of gap junctional intercellular communication (GJIC), modulation of drug- and hormone-metabolizing enzymes, activation of membrane, cytosolic and nuclear receptors, oxidative stress, and interference with intracellular transduction pathways. The cells loose their ability for homeostatic control and gain the potential for carcinogenesis.

A number of reviews concerning such mechanims have been published (Witz, 1991; Schulte-Hermann et al., 1994; Shaw and Jones, 1994; Counts and Goodman, 1995; Foster, 1997; Chevalier and Roberts, 1998). It has been

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Abbreviations: AA – arachidonic acid, AhR – aryl hydrocarbon receptor, Cdks – cyclin-dependent kinases, CYPs – cytochromes P450, ER – estrogen receptor, GF – growth factors, GJIC – gap junctional intercellular communication, LOXs – lipoxygenases, NFκB – nuclear factor κB, PGHS – prostaglandin H synthase, PPARs – peroxisome proliferator-activated receptors, pRB – retinoblastoma protein, PUFAs – polyunsaturated fatty acids, RE – response element, ROS – reactive oxygen species, TGF-ß1 – transforming growth factor beta.

shown that the carcinogenic potential of a compound is apparently associated with modulations of inter- and intracellular signal transduction pathways and cell kinetics in target tissues. Therefore, specific biochemical and cellular responses may become a useful tool for *in vitro* identification and/or quantification of specific modes of action. The aim of this paper is to shortly summarize the recent knowledge, point out key connections of the modes of action of xenobiotics, and find and assort biochemical and cellular parameters suitable for *in vitro* detection of carcinogens with the epigenetic mode of action.

Cellular communication and signal transduction

Cell behavior and homeostasis are regulated by complex mechanisms of extra-, inter- and intracellular communication. Endogenous compounds, including hormones, growth factors, and neurotransmitters, as well as various exogenous factors, such as dietary compounds, drugs, and chemicals, can act as signals which are recognized and interpreted by the appropriate cells (Trosko, 1998). Then, these cells transduce the molecular information into various second messages that trigger a number of distinct and cross-talking signal transduction pathways (Herrlich et al., 1992). These intracellular mechanisms epigenetically alter the expression of genes or gene products of the cells and can modulate GJIC. As demonstrated in Fig. 1, the modulation of cell behavior involves the following steps:

1) changes of extracellular communication and "primary" events caused by signal molecules; they include modulation of levels and disposition of endogenous hormones, cytokines, and other modulators, as well as the input of various xenobiotics and/or their intermediates, fatty acid release and oxygenation, activation/competition of membrane, cytosolic and nuclear receptors, responses including the induction of drug- and hormone-metabolizing enzymes, and enhanced production of reactive prooxidants;
2) changes of GJIC, inter- and intracellular transduction pathways, which are triggered by recognition of extracellular signals, and changes of the DNA methylation/demethylation and histone acetylation/deacetylation status; all these events may eventually alter the expression of genes and their products;
3) effects at the cellular and tissue levels affecting the cell cycle, cell proliferation, differentiation and apoptotic patterns, and adaptive responses of differentiated cells; all these processes influence homeostasis in tissues.

Epigenetic mechanisms of carcinogenesis

Extracellular signaling molecules and interactions with enzymes metabolizing drugs, hormones and fatty acids

Cytochromes P450 (CYPs) rank with the most important enzymes involved in the metabolism of xenobiotics, steroid hormones, polyunsaturated fatty acids (PUFAs), and retinoids (Waxman, 1999). These enzymes have been proposed to play key roles in the regulation of steady-state levels of endobiotics that control growth, homeostasis, differentiation, and endocrine functions (Nebert, 1991). The expression and induction of CYP enzymes are associated with activation of intracellular receptors such as aryl hydrocarbon receptor (AhR), novel orphan receptor, pregnane receptor, or peroxisome proliferator receptors (PPARs) (Waxman, 1999). The expression of many CYP genes can be transcriptionally activated by xenobiotics. An example thereof is the induction of the CYP1A isoenzymes mediated by AhR ligands, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin, coplanar polychlorinated biphenyls, several polycyclic aromatic hydrocarbons, and some other chemicals (Machala et al., 1996; Denison and Heath-Pagliuso, 1998). These events lead to an alteration of levels of bioactivating and detoxifying enzymes and increased metabolic activation of procarcinogens (Guengerich and Shimada, 1998), changes in production of eicosanoids, imbalances in concentrations of hormones and other signal molecules, and other undesirable processes such as peroxisomal proliferation and oxidative stress (Marks et al., 1995; Scarborough et al., 1999).

A modulation of enzymes of steroidogenesis, especially CYP19 enzyme (aromatase) and/or CYP11A, has been suggested as an alternative, receptor-independent mode of action of exogenous endocrine disrupters (Machala et al., 1998). However, the significance of the modulations of CYPs responsible for the biosynthesis and catabolism of steroid hormones remains unknown (Machala and Vondráček, 1998). Recent studies have shown that susceptibility to cancer, adverse effects of drugs, and effective drug treatment are often associated with polymorphism of CYPs and other drug-metabolizing enzymes (Nebert et al., 1999).

Dietary PUFAs, particularly arachidonic acid (AA) and its metabolites eicosanoids, rank with the important epigenetic factors involved in the promotion of certain types of malignancies. As diet components, they may change the cell membrane composition. After release from membrane phospholipids under the influence of various endo- or exogenous stimuli, they bind to and modulate the activity of various signaling proteins (such as protein kinase C and various mitogen-activated kinases) and serve as substrates for the posttranslational modification of the signaling proteins (DiMarco, 1995; Sellmayer et al., 1997). The enzymes of AA conversion, such as prostaglandin H synthases and lipoxygenases, rank with the important classes of drug-metabolizing enzymes (Nebert and Feyereisen, 1994). PUFAs and eicosanoids act as modulators and the second messengers of the inter- and intracellular information net controlled by cytokines and steroid hormones, and they can influence the cellular processes like proliferation, differentiation,