Table 1. Biomarkers of epigenetic toxicity of xenobiotics

Biomarker	Effect monitored in the assay	References
Concentration of signaling molecules (hormones, GF, cytokines, eicosanoids)	increase, decrease	(Sherman et al., 1992; Moos et al., 1997; Marks et al., 1998)
Drug-metabolizing enzymes (CYPs, PGHS, LOXs)	changes of activity	(Dubois et al., 1996; Eaton and Bammler, 1999; Scarborough et al., 1999)
Oxygen and nitrogen radicals, lipid peroxidation, oxy-DNA	increased production (oxidative stress)	(Klaunig et al., 1998; Radjendirane and Jaiswal, 1999; Wiemels and Smith, 1999)
Intracellular receptors (ER, AhR, PPAR)	activation/competition	(Garrison et al., 1996; Ledwith et al., 1996; Crisp et al., 1998)
GJIC	inhibition of GJIC, changes of expression and localization of connexins	(Jansen et al., 1996; Upham et al., 1998; Trosko et al., 2000)
Pathways of signal transduction (phospholipid metabolism, kinases, phosphatases, ion metabolism)	activation or inhibition	(Shawver et al., 1995; Fubini et al., 1998; Rummel et al., 1999)
Transcription factors (AP-1, NFκB)	changes of activity	(Aw, 1999; Dalton et al., 1999)
Critical gene expression (protooncogenes, tumor suppressor genes)	changes at transcriptional, translational, posttranslational levels (up- or down-regulation)	(Harris, 1996; Hasmall et al., 1997)
Cell cycle regulators (cyclins, Cdks)	changes of expression and activity	(Afshari and Barrett, 1993; Hui and Makuuchi, 1999)
DNA methylation	methylation/demethylation status	(Counts and Goodman, 1995; De Marzo et al., 1999)
Histone acetylation	acetylation/deacetylation status	(Rennie and Nelson, 1999)
Cell-cycle kinetics	changes of proportions of cells in cell-cycle phases	(Afshari and Barrett, 1993; Hader et al., 1996)
Cell proliferation	increased DNA synthesis, mitosis, cell growth	(Cunningham and Matthews, 1995; Jones et al., 1996; Foster, 1997)
Cell differentiation	inhibition, dedifferentiation	(Sell, 1993; Nilson et al., 1995)
Cell death (apoptosis, necrosis)	increase, decrease	(Schulte-Hermann et al., 1994; Roberts et al., 1997; Christensen et al., 1999)

activities at the membrane, cytosolic or nuclear levels, the alterations of which may serve as early symptoms of possible damage. Modification of structure or function of these molecules may reflect, and hopefully predict, specific and quantifiable endpoints at the cellular or higher levels. In spite of a great number of the identified signal-transduction pathways in the cell, complexity of the processes, multifacetal and fragile balance(s) in regulatory mechanisms, and "cross-talks" among individual transduction pathways, the endpoints at the cellular and/or tissue levels may be similar. The inhibition of GJIC and changes of cytokinetics seem to be integral indicators of perturbance of homeostasis in a given cell population and/or tissue (Trosko and Ruch, 1998). Such changes may cause long-term dysbalance between cell gain and loss and reflect conditions that may lead to neoplastic diseases.

Besides the tests of toxicity, genotoxicity, and long-term tests of animal carcinogenicity, there is only a limited number of generally accepted *in vitro* assays detecting the epigenetic toxicity of the agents. They include mainly cell transformation assays and determination of inhibition of GJIC (Yamasaki, 1995; Trosko et al., 2000). On the basis of recent knowledge referred to in the previous sections and using new experimental tools, it could be possible to establish a relatively unified set of

additional specific biochemical, cellular, and molecular methods detecting carcinogenic potentials (particularly epigenetic) of individual chemicals or their environmental mixtures. This approach is based on studies of mechanisms of action of the individual agent at various levels, each of them having its specific predictive value.

Table 1 summarizes candidate biomarkers for assays of adverse epigenetic effects. The first level of events includes (a) changes of drug-metabolizing enzyme activities and modulations of steroid and lipid metabolism including pathways of arachidonic acid conversion, and (b) production of oxygen- and nitrogen-reactive metabolites. The second level consists of (a) activation of intracellular receptors, inhibition of GJIC, perturbations in signal-transduction pathways, including activation or inhibition of specific kinases, phosphatases, etc., deregulation of Ca^{2+} (and other ion) metabolism, (b) changes in the expression of critical genes and proteins, i.e. protooncogenes, tumor suppressor genes, and molecules regulating the cell cycle. The third level includes biological endpoints of the different mechanisms, particularly changes in cell cytokinetics (cell cycle, cell proliferation, differentiation, and death). Particularly important are approaches and methods that enable studies of dysbalances and tendencies of related processes, like changes in the proliferation/apoptosis ratio, prooxidative/antioxidative processes, or activation/inhibition of transcription factors.

There are several important points that should be considered in the evaluation of epigenetic carcinogenic effects of the agents. It is necessary to realize that the effects are (a) mostly species- and cell type-specific, (b) dependent on the stage of development, (c) strongly dose-dependent, and (d) time-dependent. Thus, the key step is the choice of applicable experimental *in vitro* systems. The dose affects the mechanism, and mostly a long-term chronic or repeated exposure is necessary to manifest the effect of carcinogens with the epigenetic mode of action; therefore the investigations of dose- and time-dependent responses are very important (Goodman, 1998). Moreover, quantitative assessment of the summary effect is necessary (Portier et al., 1993; Burkart and Jung, 1998). As shown by our previous studies, exact mathematical processing of experimental or clinical data facilitates the understanding of interactions among the factors under study and allows assessing the predictive values of specific markers (Kozubík et al., 1997; Hofmanová et al., 1998; Kozubík et al., 1998; Hofmanová et al., 2000). Thus, it is needful to improve not only test systems, but also mathematical processing and interpretation of results. The final goal is to correlate the effects detected at the molecular and biochemical levels with the outcomes at the cellular or higher levels and to choose parameters with the highest predictive values.

In conclusion, research focused on understanding the modes of action of xenobiotics of interest is of fundamental importance for the development and validation of *in vitro* methods for the detection of carcinogenic potential. Multidisciplinary and multibiomarker approaches are necessary for setting up a battery of such methods. Such approaches will be useful not only for environmental and health risk assessment, but also for pharmacology and predictive oncology, and generally for better understanding of essential aspects of biology.

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