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Workshop 2.3

Application of toxicogenomics to the endocrine disruption issue*

Tomoyuki Shirai‡ and Makoto Asamoto

Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, 1-Kawasumi, Nagoya 467-8601, Japan

Abstract: Toxicogenomics can be expected to be a useful method for detecting the carcinogenic potential of endocrine active substances (EASs) in the short term with the generation of understanding of mode-of-action and mechanisms when a reliable database with information about proteomics and informatics is established. At present, there are no concrete epidemiological data supporting any exogenous EAS contribution to hormone-related organ carcinogenesis in humans. However, with the establishment of appropriate animal models and analysis of genomic-scale gene expression, risk identification and evaluation should be facilitated within a relatively short period, and this approach eventually promises to contribute a great deal of risk management regarding EASs.

INTRODUCTION

Over the past several years, great concern has been raised as to possible disruption of hormonal systems by environmental chemical substances. The issue of endocrine disruption (ED) has attracted the attention of scientists as well as the media. Although such chemicals were defined as "exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects, in an intact organism or its progeny, of (sub)populations" at the Weybridge Workshop, 1996, no firm criteria were adopted for assessment of adverse health effects. As far as endocrine systems are concerned, the issue is basically confined to the hypothalamus-pituitary-gonads and thyroid axis. Diverse phenotypic changes that arise from disturbance of the actions of female, male, and/or thyroid hormones through agonistic and antagonistic mechanisms or alteration of the homeostatic control balance are clearly concerns for health. In addition to their potential influence on reproductive parameters such as spermatogenesis, the sexual cycle, and development of sexual organs, they may affect processes underlying endometriosis, carcinogenesis in hormone-related organs, and neurotoxicity leading to intelligence impairment and emotional instability (Fig. 1).

The simultaneous analysis of expression of thousands of genes as endpoints using cDNA chips or microarrays should allow toxicologists a new comprehension of toxicological issues. Toxicogenomics, the combined field of toxicology and genomics, thus has become a focus for the research community and regulatory authorities as a new approach to understanding mode-of-action. It can provide us with very helpful data relevant to difficult areas such as dose–response relationships, species-to-species extrapolation, and exposure assessment that cannot be resolved with traditional toxicological techniques

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‡Corresponding author

- Reproductive dysfunction (spermatogenesis, sexual cycle, endometriosis)
- Enhancement of carcinogenesis (breast, uterus, prostate, testis)
- Immune toxicity (hyporesistance to infection)
- Developmental disorders of sexual organs (reproductive organ malformation, hypoplasia)
- Elevated carcinogenic potential in the 2nd generation (vagina, breast, uterus, prostate, testis)
- Neurotoxicity in the 2nd generation (growth retardation, intelligence impairment, emotional instability)

Fig. 1 Presumed adverse effects of EDCs.

(Fig. 2). Application of genomic technology to the ED issue can thus be expected to overcome limitations of conventional methods. One possibility is that toxicogenomics will facilitate differentiation of gene responses specific to endocrine activity from those associated with nonspecific general stress. For example, sequential analysis of gene expression profiles in endometrium with histological alterations after treatment with 17β-estradiol or related estrogenic compounds may give us estrogen-specific gene alterations, as presented by Orphanides in Workshop 2.5. As noted above, ED may lead to diverse and complex phenotypic outcomes. Since toxicogenomics is defined as elucidation of phenotypic alteration in gene expression, it causes a very broad spectrum of possibilities.

- Establish signatures of specific chemically induced altered gene expression
- Identify biomarkers of exposure and toxicity
- Predict toxicity of unknown agents
- Classify and predict phenotypes of toxicity
- Delineate models/mechanisms of action
- Allow extrapolation from one species to another (from animals to human beings)

Fig. 2 Toxicogenomics-expected promise.

Therefore, in order to make toxicogenomic approaches to the ED issue efficient, it is necessary to focus on one particular aspect for analysis. Among diverse potential effects exerted by endocrine active substances (EASs), elevation of cancer risk in endocrine-related organs is one with most concern. Thus, concentration of effort on hormone-related carcinogenesis is worthy of particular attention, because hormone-related carcinogenesis is assumed to occur by nongenotoxic mechanisms, with a long periods between exposure and appearance of neoplasms. Disadvantages with long-term animal experimentation to detect EAS-associated cancer risk may be overcome by application of toxicogenomics accompanied by relevant elucidation of mode-of-action. Predictive toxicology, i.e., prediction of toxicological adverse effects in terms of a pattern or set of genes is one important task of toxicogenomics. Whether any signature of gene alteration specific to carcinogenesis can be established remains for future elucidation.

HORMONE-RELATED CARCINOGENESIS AS A TARGET OF TOXICOGENOMICS

Carcinogenic risk associated with an EAS can be divided into three categories: (1) potential carcinogenicity of the EAS itself as seen in cases of vaginal adenocarcinomas in young girls exposed to diestilbesterol in their uteri; (2) induction of a condition predisposing to cancer development, so that it is accelerated; and (3) promotion or enhancement of cancer development by prolonged action. In line with these three modes-of-action, we must construct a database of expression profiles for gene alteration using cDNA microarray or chip technology.

There are several organs in which carcinogenic responses could be modified by an EAS. These include the breast, endometrium, prostate, testis, and ovary. For elucidation of gene expression profiles related to carcinogenic processes in individual organs/tissues, appropriate animal models are essential (Fig. 3). Carcinogenic potential for suspicious chemicals could be explored by administration of DES

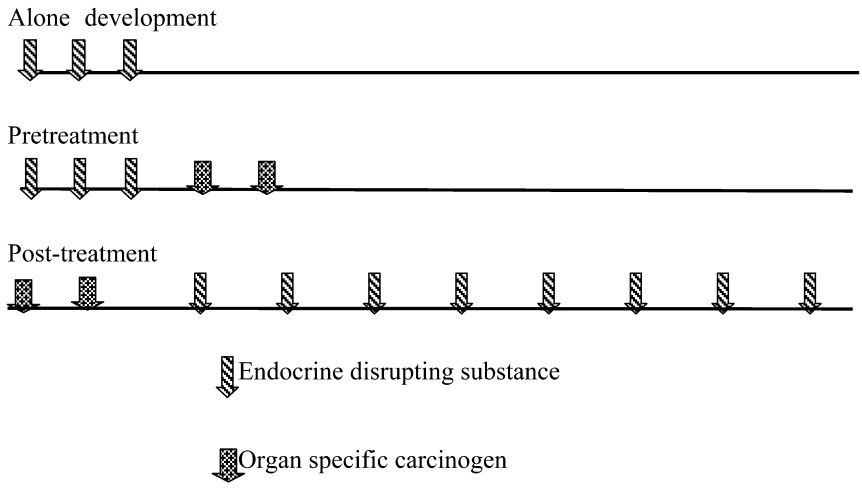

Fig. 3 Possible reference animal models.

to rats or mice [1], with tissue samples taken from the uterus, mammary glands, or vagina at certain time points for analysis of changes in alterations of expression at the genomic level. Promoting/enhancing effects of EAS could be evaluated by application of the two-stage carcinogenesis concept to the organs of interest. For mammary glands, 7,12-dimethylbenz[a]anthracene (DMBA)- or *N*-methyl-*N*-nitrosourea (MNU)-initiated rat models have been widely utilized. As a reference chemical, E2 can be given after the initiation until a sufficient yield of mammary tumors is attained [2]. During the tumor induction, changing in gene expression can be analyzed sequentially to determine whether a tumor promotion specific gene profiles exists. Similar approaches could be applied using established animal models for endometrial and prostate carcinogenesis. For the former there is a rat model with intrauterine administration of ENNG and then E2 [3], and for the latter, DMAB- or PhIP-initiated rat models are good candidates [4]. Unfortunately, at the present there are no appropriate animal models for testicular or ovarian carcinogenesis. The possibility of increased likelihood of cancer development in offspring if they were pre- or postnatally exposed to EASs is of great concern. Animal models could be envisaged with application of estrogen during gestation and lactation to mothers and then exposure of offspring to potential carcinogen at a certain point or for an appropriate period after weaning. Boylan and Calhoon [5] have already shown that prenatal exposure to DES potentiates mammary tumorigenesis in rats given DMBA. However, in spite of several attempts, in utero and lactational exposure to bisphenol A or 4-nonylphenol did not produce a predisposition for cancer development in the prostate or uterus [6]. It can be expected that a database of gene profiles associated with EASs will become available as and only when appropriate and reproducible animal models are established.

Confidence in results might be elevated by combination with traditional toxicological and toxicopathological findings. Evaluation of any influence on cancer development in hormone-related organs after early life exposure to endocrine disruptors is likely to be very difficult, because there is little knowledge about susceptibility. If cancers do develop due to exposure to environmental chemicals, genes associated with chemical activation or detoxification, DNA repair genes, and genes associated with cellular replication might be targets. In addition, genes enconding hormone metabolic enzymes such as 5α-reductase and aromatase, expression of hormone receptor-related and coactivator genes are obvious candidates for assessment.

In Japan, a consortium project on toxicogenomics for carcinogen detection has just commenced. This project was initiated by the Ministry of Economy, Trade and Industry and is supported by a grant from the New Energy and Industrial Technology Development Organization. As reference compounds, quaternary categorized chemical carcinogens in the liver such as mutagenic or nonmutagenic hepato-

carcinogens and mutagenic nonhepatocarcinogens and noncarcinogens are administered to rats for short periods and database of gene expression profiles is being constructed with tissues sampled at various time points using in-house cDNA microarrays. Proteomic studies are also carried out at the same time. Once carcinogenesis-related genes are selected from nonspecific altered genes, the information should contribute to understanding at mechanisms of EAS-associated carcinogenesis.

Promotion or antipromotion effects of possible EASs could be assessed by evaluation of expression profiles of genes involved in cell replication such as cyclines, cycline-dependent kinases (CDKs), and CDK inhibitors, as well as their counterparts associated with cell death pathways and cell differentiation. It can be envisaged that efforts with specific organs like the mammary glands, endometrium, prostate glands, and thyroid, which depend upon hormonal activities, will be most rewarding.

CONCLUSION

Toxicogenomics can be expected to be a useful method to detect the carcinogenic potential of EASs in the short term with generation of understanding of mode-of-action and mechanisms when a reliable database with information about proteomics and informatics is established. At present, there are no concrete epidemiological data supporting any exogenous EAS contribution to hormone-related organ carcinogenesis in human beings. However, with establishment of appropriate animal models and analysis of genomic-scale gene expression, risk identification and evaluation should be facilitated with a relatively short period, and this approach eventually promises to contribute a great deal of risk management regarding EASs.

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