[14:10 - 14:50]

Toxicogenomics in relation to biomarkers: Current status and future aspect

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Safety evaluation of pharmaceutical products has been conducted in the form of risk assessment and the study of prediction and prevention of adverse effects on the basis of extensive scientific area over toxicology, pathology, pharmacology, biochemistry, physiology, etc. With the rapid progress of genomic science, diseases and their causes have become understood at the genetic level. With remarkable advancement both in drug discovery, R&D activities and in concomitant technical supports, drug discovery strategies have become focused on drug safety screening in their early stages. Today, drug discovery efforts start from "molecular targeting" based on pharmacogenomics, probably because the introduction of drug discovery research centered on gene targeting as well as combinatorial chemistry has realized synthesis of many compounds in a short period. The phenomenon suggests the necessity of evaluating toxicity of various compounds with small amounts of their samples as quickly as possible. This series of toxicological strategies is now commonly called "High-Throughput Toxicology (HTP-Tox)," an essential part of toxicological study in the early phase of drug discovery.

To have a sufficient safety assessment and its evaluation, introduction of the appropriate toxicologically responsible biomarkers are indispensable. In vitro and in vivo evaluation systems, particularly as screening systems, play an important role, in the early phase of pharmaceutical development. They are also important to clarify the mechanisms of toxicity observed during development. On the other hand, toxicopanomics technologies (a collective designation for the "-omics" such as

toxicogenomics, toxicoproteomics and metabonomics) is expected to be applicable to predictive toxicology and mechanism-based risk assessment in the area of toxicology (Stubberfield et al., 1999, Pennie et al., 2000, Suter et al., 2004). Currently, toxicopanomics technologies are being applied to the development of new safety evaluation systems.

Biomarkers in gene expression / regulation in toxicity generation

As a general concept of biological response, the resulted activities are recognized as normal, pharmacological, and toxicological effects under the exposure of xenobiotic (compound) to the target cell. In any case, gene-expression is concerned for the induction of related reaction. In the toxicity generation, direct, indirect or regulative effects through the target gene cause the toxicological reactions. (Fig. 1)

Toxicopanomics aspect for investigation of new biomarker

Generally, to the processes through gene polymorphism in genome (DNA level), gene expression in transcriptome (RNA level), protein synthesis in protein (proteome), and metabolism in metabolome, toxicologically responsible biomarkers are newly focused as a gene-related biomarker. In terms of the detection / estimation of these biomarkers in the expression process, timing of on-set and sustainability are to be considered as an important factor. In addition, the information is to be addressed to the drug susceptibility / sensitivity in individual drug-treatment. (Fig. 2)

Case study 1: Toxico-genomics / -proteomics approach in hepatotoxicity

Four compounds known as hepatotoxicity were investigated (Kikkawa et al., 2006, Kikkawa et al., 2005, Yamamoto et al., 2005, Yamamoto et al., 2006): Acetaminophen (APAP), Amiodarone (AMD), Tetracycline (TC) and Carbon tetrachloride (CTC). To evaluate hepatotoxicity in rats, 300 or 1,000 mg/kg of APAP, 300 or 1,000 mg/kg of AMD, 600 or 2,000 mg/kg of TC and 0.3 or 1 mL/kg of CTC were orally administered

once to rats, and changes in blood biochemical parameters as well as histopathological changes were investigated 6 and 24 hours after administration. For the livers of APAP-administered rats, changes in protein expression were investigated by proteomics. It was found 24 hours after administration that all the compounds had caused histopathological changes such as inflammatory ones. Immunohistological examinations revealed the expression of oxidative stress-related proteins 6 hours after administration. The changes in several biomarkers are the oxidative stress-related and mitochondrial metabolism-related proteins, suggesting their usefulness as hepatotoxicity evaluation markers in in-vitro / in-vivo systems. From the toxicoproteomics aspects, the common protein expression in hepatotoxicants are defined in relation to cell death, cellular assembly / organization, and lipid / carbohydrate / amino acid metabolism. (Table 1)

Case study 2: Toxicopanomics evaluation in vasculitis

Toxicopanomics (toxicogenomics/toxicoproteomics/toxicometabolomics) approach was made in the case of vasculitis. Using the vasculitis model in rat liver, clinical pathological and histopathological examinations were carried out with the analysis of gene expression, protein expression and metabonomics. In this study, compound X known as vasculitis was investigated. The clinical pathological and histopathology data suggested vascular lesions induced by compound X. Changes in gene expression, protein expression and metabolite were also found. As a result, several key parameters as a biomarker were defined in each omics approach. Combining with these biomarkers, concordance in three analyses was defined as a meaningful combined biomarker. On the whole, combinational estimation from the points of panomics analysis would be one of powerful approaches for setting the reliable biomarkers in the toxicology field. (Fig. 3) In addition, for the estimation of human relevance, gene- / protein- expressions were investigated by using human umbilical-vein endotherial cell.

In this culture system, changes in gene expression and protein expression induced by compound X were found. These panomics data can be sources of new biomarkers as well as provide insight into the mechanisms of human vascular injury. These results also contributed to development of high throughput screens to improve selection of compounds for drug development.

Future aspect

As comprehensive analyses of genes or proteins became available, bioinformatics (information-processing technologies) has long been expected to grow to interpret floods of data created by those analyses. Bioinformatics is currently under energetic study and development as technologies to select and compile data characteristic of specific life phenomena. However, trends toward the next generation have already been found; i.e., system biology, a novel study area aimed to understand life phenomena as a system (Kitano, 2001), is attracting attention. With the progress of development and implementation of new technologies represented by panomics, understanding of components constructing life such as genes and proteins has rapidly advanced. System biology is intended to comprehend such information in the dynamics of life phenomena. Results from study on system biology will assume important roles in developing simulation models such as E-cell (Tomita et al., 1999) and analyzing life behaviors including pathological conditions. The area of toxicology will also greatly benefit from system biology, which is expected to grow into "system toxicology." In the study on system biology, in-vitro experiments are still an essential process. Cell-based assay systems capable of comprehensive analysis seem to be needed to demonstrate life phenomena (hypotheses) simulated by different methods.

As described above, while development of in-vitro safety evaluation systems is dramatically advancing, information has become batch-processed with the advent of high-throughput, and comprehensive analysis systems. In the future, even in-silico

systems simulating life phenomena and automatically analyzing life behaviors will be developed. Toxicologists can benefit from such novel technologies. Now that various new tools are available to researchers, their ability and sensitivity to achieve extensive, higher-quality toxicity evaluation utilizing those tools will be tested. (Fig. 4)

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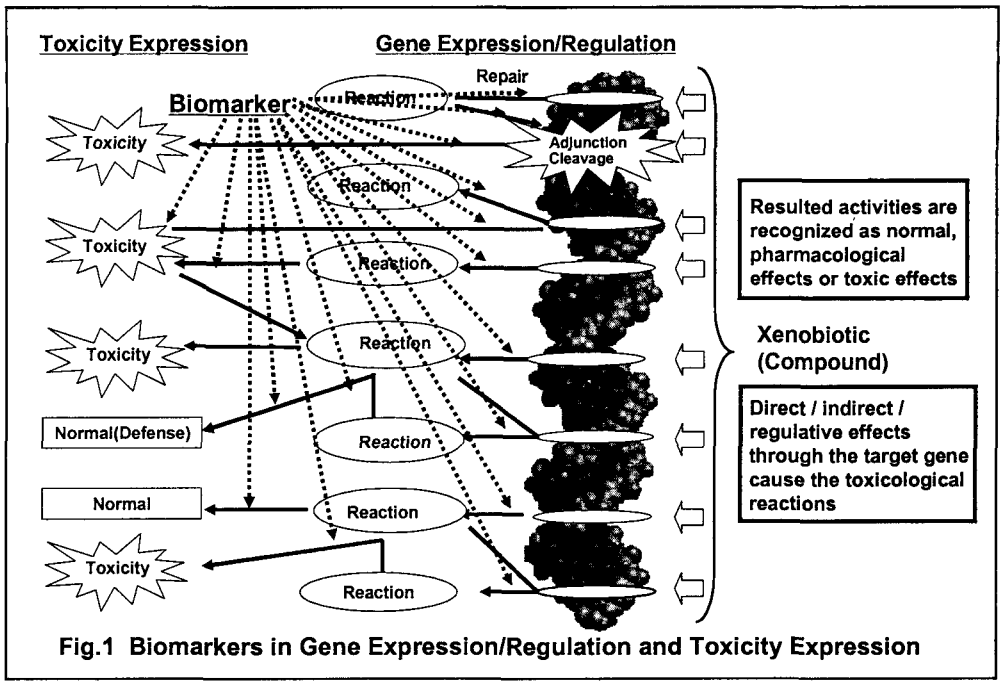

Fig.1 Biomarkers in Gene Expression/Regulation and Toxicity Expression

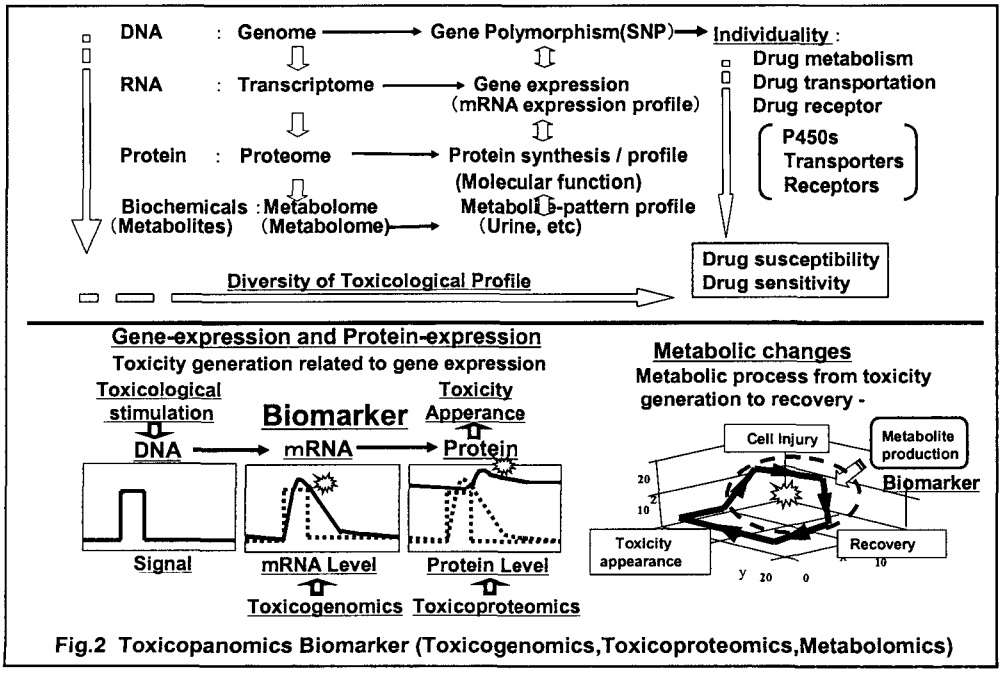

Fig.2 Toxicopanomics Biomarker (Toxicogenomics,Toxicoproteomics,Metabolomics)

Table 1 Common Protein Expression in Hepato-toxicants (APAP,AD,TC,CTC)

Cell Death

- *α-enolase* (P04764)
- *Peroxiredoxin 1* (Q63716)
- *Regucalcin* (Q03336)
- *Thioredoxin* (P11232)
- *Glutathione peroxidase* (P04041)

Cellular Assembly/Organization

- *Fructose-bisphosphate Aldolase B* (P00884),
- *Keratin 8* (Q10758),
- *Transthyretin* (P02767),
- *Calreticulin* (P18418),
- *Thiosulfate sulfurtransferase* (P24329)

Lipid Metabolism

- *Apolipoprotein A-I* (P04639)
- *Apolipoprotein A-IV* (P02651)
- *Apolipoprotein E* (P02650)
- *Acyl-CoA synthetase* (P18163)
- *α-2-macroglobulin receptor-associated protein* (Q99068)

Lipid/Carbohydrate Metabolism

- *ATP citrate lyase* (P16638)
- *Aldehyde dehydrogenase 2* (P11884)
- *Transaldolase 1* (Q9EQS0)

Amino Acid Metabolism

- *Methionine sulfoxide reductase A* (Q923M1)
- *Transthyretin* (P02767)

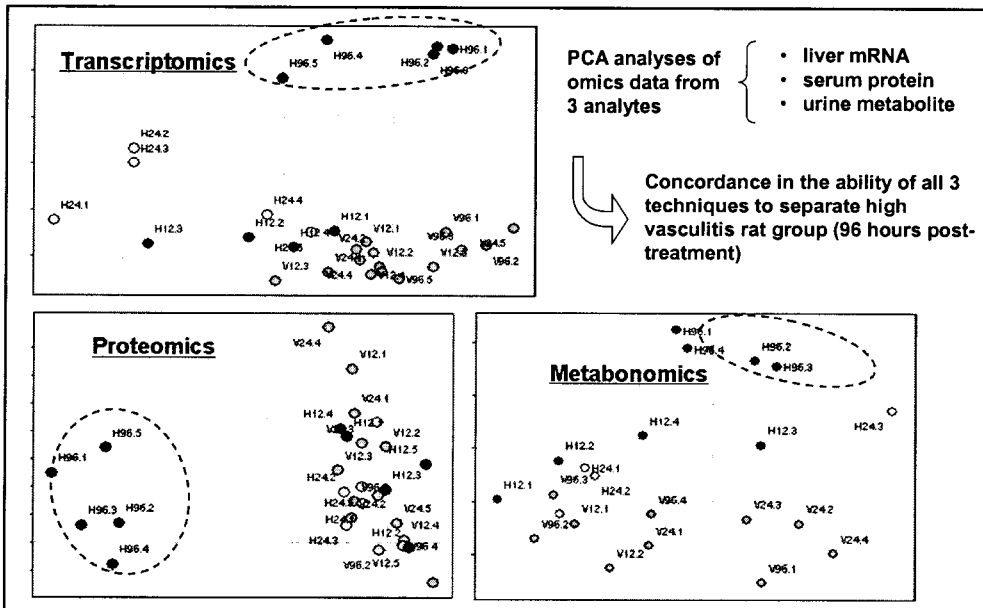

Fig. 3 Applying a "Panomics in Vasculitis: Approach to Biomarker Identification

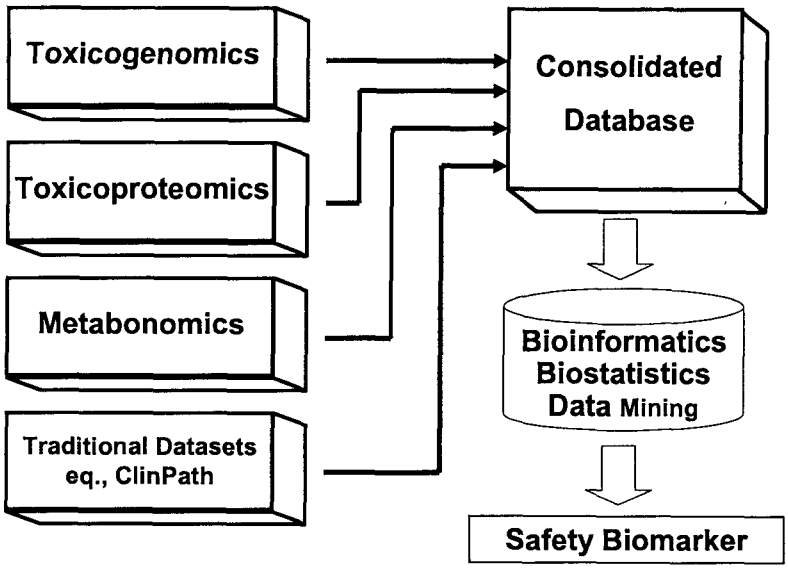

Fig. 4 Consolidation of Exhaustively Analyzing Data