Percellome toxicogenomics project and its possible contribution to 3R's

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Abstract
Percellome Toxicogenomics Project has been launched to develop the comprehensive gene cascade database/informatics for the mechanism-based predictive toxicology. For an effective accumulation of transcription data, a normalization method designated as "Percellome" is developed. By this method, mRNA expression values are given as "copy numbers per one cell" from microarrays. Thus, data are ready for direct compared among studies, different organs, and different platforms that adopt Percellome method.

Up to now, the time- and dose-dependent alteration of gene expression induced by a single oral exposure in mouse liver (4 time points x 4 dose levels, triplicate, 48 per chemical) are studied on more than 90 chemicals. Each chemical data is expressed as a 3-D graph (time x dose x copies per cell) made of 45,000 surfaces corresponding to the probe sets of the Affymetrix GeneChip MOE430 2.0 ("MilleFeuille" data, cf. http://toxicomics.nihs.go.jp/db/). Now, the project includes repeated dosage, multiorgan linkage, inhalation toxicogenomics, and fetus toxicogenomics for developmental toxicology. This mechanism-based approach should contribute to 3R's in the near future by miniaturizing the in vivo experiments and help designing new alternative methods, and should lead to our ultimate goal of creating "virtual mouse" in silico in the future. (supported by MHLW grants and others)

Keywords: toxicogenomics, molecular toxicology, gene expression cascade, percellome normalization method, 3 dimensional surface data

Introduction

Toxicogenomics monitors the effects of xenobiotics to the host as transcriptome. To fully develop the molecular toxicology including the ability to answer problems such as species differences, individual differences, and combined exposure, it is essential to reveal the entire gene expression cascade (Fig. 1). And when the histopathological changes are monitored, the molecular events are considered to be quite near to the end of the gene expression cascade. Therefore, we adopted an approach to collect transcriptome data from when there are no morphological changes yet, i.e. when phenotypic anchoring is not possible (Fig. 2). Allegorically, it is just like when electron microscopy was invented and new figures of intracellular organella were presented. It took years to understand what those new structures are. It was after a considerable amount of images have been accumulated and analyzed (Fig. 3). Likewise, for toxicogenomics, a certain amount of data has to be accumulated over a period of time. To facilitate the analysis of accumulated transcriptome data, we have developed the normalization method designated as Percellome, which normalize against the cell number of the sample, or, to generate the copy numbers of mRNAs in a "per one cell" basis. This method allows us to directly compare transcriptomics data among samples, experiments, organs, and different platforms as long as it generates Percellome data. We have

<table>
<thead>
<tr>
<th>Percellome Projects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aim:</strong></td>
</tr>
<tr>
<td>Develop Gene Cascade Database by Phenotype-Independent Approach for predictive mechanism-based toxicology</td>
</tr>
<tr>
<td><strong>Ultimate Goal:</strong></td>
</tr>
<tr>
<td>Virtual mouse, virtual human in silico</td>
</tr>
<tr>
<td><strong>Tentative Goal:</strong></td>
</tr>
<tr>
<td>High-Resolution, Mechanism-based Toxicology to reinforce Traditional Toxicology</td>
</tr>
<tr>
<td><strong>Practical Contribution:</strong></td>
</tr>
<tr>
<td>Cheaper, Faster, and More Accurate Assessment And yet Comprehensive to meet the regulatory needs (safety assessment).</td>
</tr>
</tbody>
</table>

Fig. 1. Aim, goals and practical contribution of the Percellome Projects.

The aim of the project is to develop a gene cascade data base in a comprehensive manner. Ultimate goal is to develop virtual mouse and human in silico.
Jun Kanno

utilized this method and accumulated transcriptome data for murine liver and other organs of over 90 chemicals, including drugs, pesticides, food-related chemicals, environmental chemicals and typical toxins. With the aid of a series of original software, the analysis is proceeding on the 3-dimensionally visualized data, which is biologist friendly.

Methods

Animal experiments

C57BL/6 Cr Slc (SLC, Hamamatsu, Japan) mice maintained in a barrier system with a 12 h photoperiod were used in this study. Twelve week-old male mice were given a single dose (vehicle control, low, medium and high doses, with a ratio of square root of 10) of the test compound by oral gavage, and the liver was sampled at 2, 4, 8 and 24 hours post-gavage (total of 16 groups, 3 animals per group, individually subjected to transcriptome measurement). Animals were euthanized by exsanguination under ether anesthesia and the target organs were excised into ice-cooled plastic dishes. Tissue blocks weighing 30 to 60 mg were placed in an RNase-free 2 ml plastic tube (Eppendorf GmbH., Germany) and soaked in RNAlater (Ambion Inc., TX) within 3 min of the beginning of anesthesia.

Percellome method

Details of the method are reported elsewhere (Kanno, 2006). Briefly, the tissue blocks kept overnight at 4°C in RNAlater was replaced with RLT buffer (QIagen GmbH., Germany), and homogenized. Aliquots of the homogenate were measured for DNA content by PicoGreen fluorescent dye (Molecular Probes Inc., USA) system. Then the grade-dosed spike cocktail (GSC) made of five Bacillus subtilis RNA sequences was added as dose-response standard of each sample.

Percellome data base

For male murine liver, more than 90 chemicals were tested and the 4x4 formatted Percellome data are accumulated. As the data are given as copy numbers per cell, all data form one study can be plotted as a 3-dimensional surface graph containing ca. 45,000 layers of surfaces each corresponding to the probe set of Affymetrix GeneChip MOE 430 2.0 (Affymetrix Inc.).

Fig. 3. Toxicogenomics is like electron microscopy.
New images given by the electron microscopy are hard to link to light microscopic views. And it took years to understand the ultrastructure of our body. Likewise, toxicogenomics offers a new level of data.

Millefeuille data (MF surface data)

We monitor early transcriptomic responses in time- and dose-dependent manner, visualizing each genes (probe sets) in a 3-dimensional surface. One study generates about 45,000 layers of surfaces when Affymetrix GeneChip system is used (Millefeuille data). The studies are run in triplicate so that mean and +/- 1 S.D. surface can be drawn for each gene (probe set). The surface gives a biologist-friendly view of biological responses.
Results

The 3-dimensional expression of the Percellome data turned out to be very friendly to biologists. As shown in Fig. 4, the time- and dose-dependent alteration in transcriptome of a certain gene (probe set) can be easily assessed by the 3-D surface expression. As mRNA synthesis takes place in tens of minutes to hours in order, and the dose-response relationship should be monotonic or at most concave or convex in shape within the current dose setting scheme, changes in time-dose axes are expected to be rather smooth. And the triplicate data generate mean surface and two additional surfaces corresponding to +1 and -1 S.D. When the mean surface is rough and S.D. surfaces are widely separated, the data is very likely to be either biologically less meaningful, or noise.

Fig. 5. Percellome Projects as a part of MHLW’s toxicogenomics projects
The five-year rat project now in NIBIO, Osaka was designed based on the Percellome Projects. Mouse Percellome Projects maintained in NIH now covers various areas indicated.

Discussion

It is important to distinguish two types of in vitro or alternative methods for replacing in vivo toxicity studies. One type is a "miniature black box" type. It is an in vitro version of in vivo studies in which animals are handled as black boxes, concerning chemicals as input and monitoring symptoms as output, without knowing the precise events taking place in the body. This type of alternative method, such as monitoring viability of the cells in vitro, or diminishment of a particular function of the cells in vitro needs endless validation. In other words, the validity of the method is only within the domain of the teaching studies performed and validated, or only good for interpolation. The other type is a "mechanism-excision" type, which excises out a well established biological mechanism as an in vitro alternative method. One of the most famous methods is the Ames Test. It has postulated mechanisms and positive controls. Estrogen receptor transcription assay is another good example, with estradiol as a positive

The Percellome toxicogenomics project now expands to multiorgan linkage (liver, lung, kidney, heart, testis, brain (hippocampus, cerebrum, brain stem, cerebellum), repeated dosage (14 days + single exposure), inhalation (lung, liver, 2hr single exposure, 6 hr x 7 days, and 22 hr x 7 days exposure), fetus/developmental (whole embryo, ES cells, Embryoid body), and neuro-behavior/brain (hippocampus, cerebrum, brain stem, cerebellum) (Fig. 5). The analysis on the Percellome data reveals its nature of high-resolution toxicology especially when monitoring the events well before morphological changes are induced. In the case of inhalation studies where very low "sick building"-level exposure is performed, it has proven that, even in this low level where no histological changes are induced, the lung disease-related genes are induced.

Fig. 6. Two Types of Alternative Methods
It is important to distinguish "in vivo mechanism Excision Type" alternative methods from "Miniature Black Box Type" methods.

Fig. 7. 3Rs and Mechanism-based Toxicology (ToxicoOmics)
ToxicoOmics can contribute to 3Rs in various ways and levels.
control. This type of methods can be validated by a limited numbers of positive and negative controls. Moreover, the method can extrapolate as long as the mechanisms hold.

Percellome toxicogenomics is a high-sensitivity and robust method so that its use of animal is, and will be much less than traditional toxicology studies. And above this, it will provide us with the mechanisms of various toxicity endpoints. This information should greatly facilitate designing and developing new alternative methods of "mechanism-excision" type (Fig. 7).

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Reference