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Prediction of Hepatotoxicity of Drugs Based on the Japanese Toxicogenomics Project Database

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The Toxicogenomics Project is a 5-year (2002-2007) collaborative project by the National Institute of Health Sciences, the National Institute of Biomedical Innovation, and 16 pharmaceutical companies. Its aim is to construct a large-scale toxicology database of transcriptome(mainly liver; using Affymetrix GeneChip) for prediction of the toxicity of new chemical entities in the early stage of drug development. About 150 chemical compounds were selected, and more than 100 compounds, covering wide medication categories, have been completed by September 2005. We have in vivo gene expression data (N=3 for each point) multi-time point (4 points each for single and repeated dosing) and multi-dose (4 dose levels including vehicle control) with traditional toxicological data (N=5 for each point). Statistical techniques are usually powerless for analyzing vast numbers of comparisons with few measurements, such as microarray data. We have now realized that our multi-point, multi-dose protocol is quite good at detecting toxicologically meaningful changes from data filled with noise. We have extracted various gene lists by employing the Prediction Analysis for Microarrays (PAM) to classify chemicals based on their toxicological phenotypes, pharmacological properties, etc. Gene lists characterizing different toxicological pathways and functions are now accumulated in the database and prediction of hepatotoxicity may be possible by combining them. For bridging between rats and humans, we perform in vitro tests using rat and human primary cultured hepatocytes with 3 time points and 4 dose levels (N=2). It is obvious that the simple orthologous linage of genes is fruitless, as there appear to be genes whose expression is different between in vivo and in vitro, different between species, and similar or common between rat and human. Some representative cases of these will be discussed in the presentation.

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Outline of the Project

Final goal: To construct a system which will predict the toxicity of new chemicals in the early stages of drug development.

Method: To create a large-scale database of >150 drugs on rats

Jun. 2002 - Mar. 2007 (5 year project)

Apr. 2010: Database will be open to public.

Budget: 5,000,000,000 yen ($42million) Half of the amount is from the national budget, while the remaining half is from 16 companies.

Project Core: (- Mar. 2005) National Institute of Health Sciences (Tokyo, Japan)

Project leader: T. Nagao, Sub-leader T. Urushidani & T. Miyagishima

Companies enrolled: Astellas, Chugai, Daiichi, Dainippon, Eisai, Kissei, Mitubishi-Pharma, Mochida, Ohtsuka, Ono, Sankyo, Sanwa, Shionogi, Sumitomo, Takeda, Tanabe.

Location: (Apr.2005-) National Institute of Biomedical Innovation (Osaka, Japan)

Features of the Project

1) The quantification of "absolute content of mRNA per one cell (Percellome)" is attained by using the Affymetrix GeneChip. Extensive quality control is performed.

2) The total group of 150 compounds include considerable number of "the drug of which clinical trial was terminated or of which marketing was ceased due to the fact that human toxicity emerged, even though the presence of toxicity was undetected or neglected in non-clinical tests".

3) Various toxicological data with high quality have links to the gene expression data in the database.

"Efficient experimental protocol"

Experimental Protocol: in vivo

- **Animals**
  Sprague-Dawley male SPF rats, 6 weeks

  Accumulated huge toxicological data are available.
  Whole genome information will be available soon.

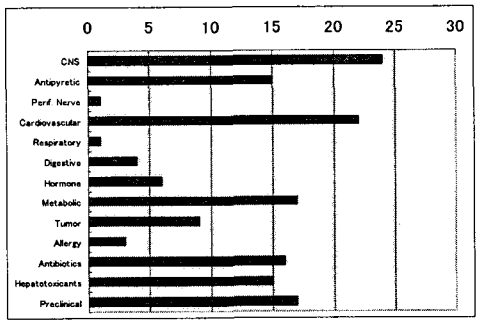
- **Target Organ**
  Liver, Kidney

- **Test items**
  Hematology, Blood Biochemistry,
  Histopathology, Organ weight, Body weight

Experimental protocol in vivo

- Sprague-Dawley male SPF rats, 6 weeks
- Target organ: Liver (150) Kidney (30)
- Test items: Hematology, Blood Biochemistry, Histopathology, Organ Weight, Body Weight, etc. (N=5)
- Affymetrix GeneChip (N=3)
- Single dose: 3, 6, 9, 24 hr
- Repeated dose: 3,7,14,28 days
- vehicle + 3 doses

Chemicals Selected (150 compounds)



Examples of analysis

1. Vehicle control, circadian genes
2. Merit of multi-dose, multi-time protocol
3. Mechanism-based analysis
4. Predictive toxicogenomics

Example 1. Vehicle control GeneChip RAE230A

Target Organ: Liver

Single Oral Administration: 0.5% MC or Corn oil

Sacrifice: 3, 6, 9, 24 hr after dosing

-> Circadian genes could be detected.

Repeated Oral Administration

For 3, 7, 14, 28 days (in the morning)

Sacrifice: 24 hr after the last dosing

-> Genes differentially expressed with age could be detected.

Effect of vehicle: MC vs. Corn Oil

- It was desirable that the vehicle for suspending drugs was unified to be methylcellulose.
- However, there are many test compounds with poor dispersibility, and strong detergents or organic solvents are undesirable because of their potent bioactivity.
- We therefore inevitably chose corn oil as a vehicle for the highly hydrophobic compounds.
- 5 ml/kg corn oil corresponds to about 11% of the total calories. Moreover, this is administered once in the morning when the feeding behavior of the rat is normally inactive.

Life Sci. in press

Corn oil-modified genes [redacted] related to lipid metabolism

1. CYP7a1 rate-limiting enzyme of bile acid synthesis
2. CYP8b1 controls the ratio of cholic acid over chenodeoxycholic acid
3. HMGCoA reductase cholesterol biosynthesis
4. angiopoietin-like protein 4 Involved in lipid metabolism via inhibition of lipoprotein lipase activity
5. Aldehyde dehydrogenase1A1
   Epoxide hydroxylase 2 [redacted] detoxication of metabolites from lipid metabolism
6.
7.
8.

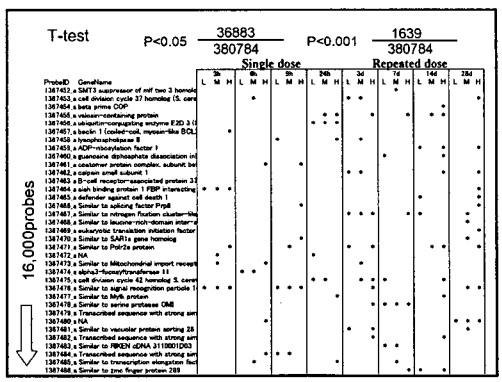
Most of them were considered to be favorable for high lipid diet.

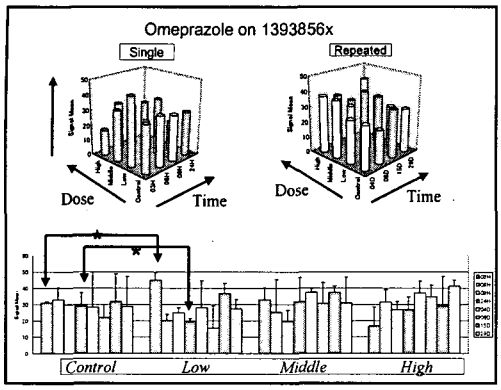
Example 2. Merit of multi-dose, multi-time protocol

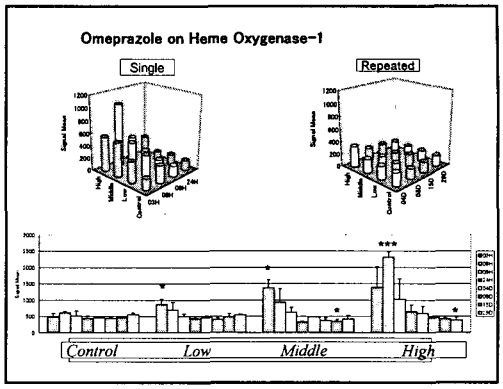
- Omeprazole 100, 300, 1000mg/kg p.o. (low, middle, high)

Single: 3, 6, 9, 24 hr

Repeated: for 3, 7, 14, 28 days







- Acetaminophen 50, 1000mg/kg p.o.
- 6 weeks vs. 12 weeks

12 week old rat was found to be more sensitive than 6 week old
-> Why?

Genes differentially expressed between 6W and 12W at 24 hr.

They contained HSP70, Heme oxygenase 1, etc.

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Multiple time and dose protocol revealed that their expressions appeared to be different because their peak, not the extent, of expression was different between ages.

Example 3. Mechanism-based analysis

- In addition to the chemicals listed, several heptatotoxicants were tested in order to accumulate knowlege about the toxicological pathway.

Glutathione depletors, TNFα/LPS, ER-stressor, protein synthesis inibitor, etc.(single dose protocol)

Phorone : Glutathione depletor

Extraction of Glutathione-depletion marker

- Extract genes negatively correlated with hepatic glutathione contents
- Confirmation by Principal Component Analysis
- Hepatotoxicants depleting glutathione are extracted from the database.

4. Predictive toxicogenomics

Strategy

Phenotype anchoring tool
Clustering tool
Published information

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Gene list manager ⇔ Marker gene lists

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Prediction Analysis for Microarrays (PAM)
Support Vector Machine (SVM)

Example : methapyrilene and thioacetamide
"nongenotoxic carcinogen"

Procedure

- Extraction of dose-dependently modulated genes by K-means clustering
- Selection of genes commonly modulated between methapyrilene and thioacetamide
- Perform PAM to obtain optimal discriminator
- Scoring the results by PAM

The non-genotoxic carcinogenicity of the chemicals in the database was successfully quantified

Conclusion

1. In The Toxicogenomics Project, a large scale database of >150 drugs with sufficient quantity and high quality of data is being constructed.
2. These data would be useful for analysing toxicological mechanism of hepatotoxicants.
3. A combination of mining tools with discriminant analyses would be effective for predicting toxicogenomics.

Toxicogenomics Project

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Sub-leader: Toshikazu Miyagishima, Tetsuro Urushidani
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