【S-5】

Toxicogenomics in Drug Discovery: Predictive Toxicology

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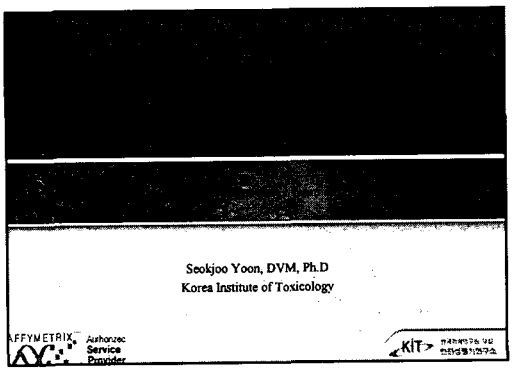
Human genome project의 종료선언과 함께 생명과학분야는 굵직한 변화를 겪게 된다. 그중 하나가 genomics라는 분야다. Genomics는 기초생명분야 뿐만 아니라 약리, 독성 등의 응용분야에 까지 두루 활용되고 있다. 그중 신약개발과 안전성평가와 관련하여 살펴보면 다음과 같다. 2005년 초에 미국 FDA는 pharmacogenomics를 이용한 약효관련 자료 제출에 관한 공식적인 가이드라인을 발표했다. 의무적 제출사항은 아니지만 참고자료로서 제출이다. 하지만 이러한 변화는 초기에 genomics 기술의 신약개발 및 독성평가연구에의 응용에 소극적이던 분위기를 반전시키기에 충분한 것이었다. 대형제약회사를 중심으로 이러한 자료를 준비하여 적극적으로 신약후보물질의 약효 및 독성평가분야에 활용하고 있다. 안전성평가분야에서도 genomics의 응용은 하루가 다르게 발전해가고 있다. Toxicology와 genomics가 합쳐진 toxicogenomics(독성유전체)라는 분야도 빠른 발전을 해나가고 있다. Toxicogenomics의 가장 큰 장점인 대량의 정보를 단시간 내에 얻을 수 있다는 점은 high throughput system (HTS)와 잘 연결이 된다. 신약개발의 측면에서는 독성평가 수준이 분자생물학적 기전의 수준에 이를 것이며 high throughput 안전성 스크리닝 및 표적 독성 스크리닝이 효율적으로 수행되기 위해서는 독성유전체 기술이 활용될 것이다. 이를 통하여 독성으로 인하여 발생하는 문제를 분석하고 신약개발 의사결정이 가능하게 될 것으로 예상된다. 안전성평가의 측면에서는 장기간 실험을 요하는 발암성 시험 등의 기간을 단축시키고자하는 노력이 독성유전체연구를 통하여 이루어지고 있다. 이는 molecular signature라는 유전자 발현 패턴의 분석을 통한 특정유전자의 발현을 통한 독성물질의 발암성 여부를 예측하는 것이다. 현재 다각적인 접근 방식을 통해 연구가 이루어지고 있다. 안전성평가에서 또 하나 빠질 수 없는 것이 바로 새로운 biomarker의 발굴이다. 간독성, 신장독성, 신경독성등의 장기 특이적 독성물질의 biomarker를 개발하여 신약후보물질의 독성을 조기 스크리닝하는 예측독성학(predictive toxicology)으로의 활약도 예상되어진다. 최근 BT와 IT기술의 융합이 가속화되는 가운데 in silico toxicology에 대한 기대도 높아지고 있다. 이것은 생물학적 정보를 활용하여 컴퓨터 시뮬레이션으로 가상생체(인체)에서의 독성물질/화학물질의 체내동태 및 안전성을 보다 정확하게 예측하는 것이다. 향후 미국 FDA는 신약의 허가 신청시 인체모델에서의 안전성 시험결과를 요구할 것으로 전망되고 있어 관련 기술의 발전이 시급한 상황이며 genomics관련 기술의 활용이 기대되어진다.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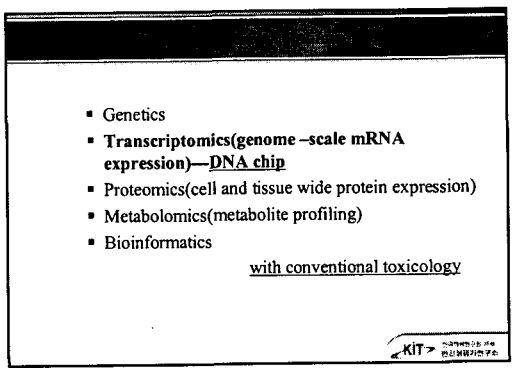
Genetics
Transcriptomics(genome –scale mRNA expression)—DNA chip
Proteomics(cell and tissue wide protein expression)
Metabolomics(metabolite profiling)
Bioinformatics
with conventional toxicology
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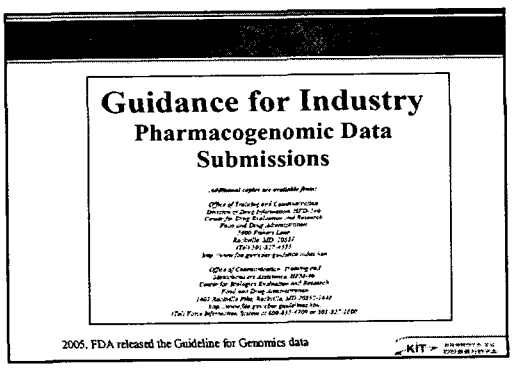
Guidance for Industry
Pharmacogenomic Data Submissions
2005. FDA released the Guideline for Genomics data
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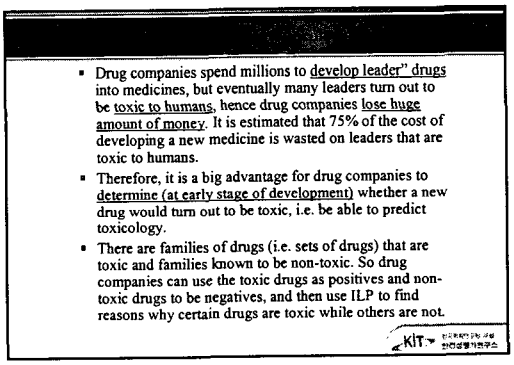
Drug companies spend millions to develop leader" drugs into medicines, but eventually many leaders turn out to be toxic to humans, hence drug companies lose huge amount of money. It is estimated that 75% of the cost of developing a new medicine is wasted on leaders that are toxic to humans.
Therefore, it is a big advantage for drug companies to determine (at early stage of development) whether a new drug would turn out to be toxic, i.e. be able to predict toxicology.
There are families of drugs (i.e. sets of drugs) that are toxic and families known to be non-toxic. So drug companies can use the toxic drugs as positives and non-toxic drugs to be negatives, and then use ILP to find reasons why certain drugs are toxic while others are not.
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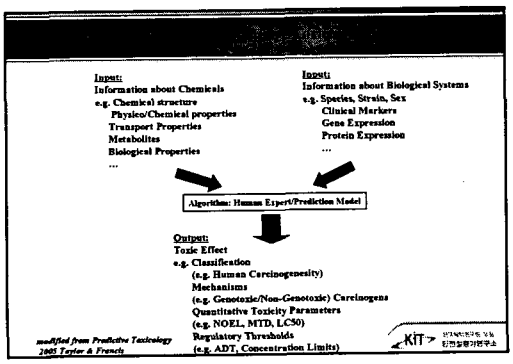
Input:
Information about Chemicals
e.g. Chemical structure
Physico/Chemical properties
Transport Properties
Metabolites
Biological Properties
...
Input:
Information about Biological Systems
e.g. Species, Strain, Sex
Clinical Markers
Gene Expression
Protein Expression
...
Algorithm: Human Expert/Prediction Model
Output:
Toxic Effect
e.g. Classification
(e.g. Human Carcinogenesity)
Mechanisms
(e.g. Genotoxic/Non-Genotoxic) Carcinogens
Quantitative Toxicity Parameters
(e.g. NOEL, MTD, LC50)
Regulatory Thresholds
(e.g. ADT, Concentration Limits)
modified from Predictive Toxicology 2005 Taylor & Francis
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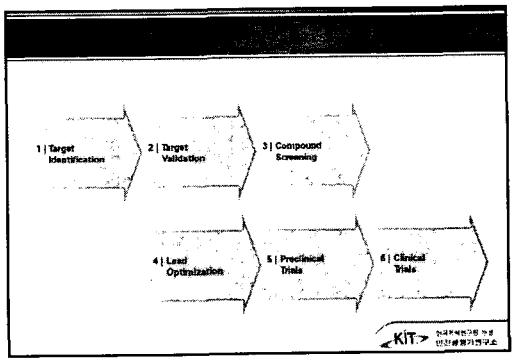
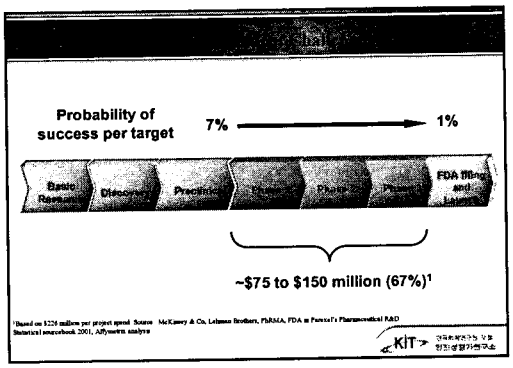

1 | Target Identification
2 | Target Validation
3 | Compound Screening
4 | Lead Optimization
5 | Preclinical Trials
6 | Clinical Trials
KIT



■RNA transcription Analysis
-understanding a compound's risk profile earlier in the development process should allow more efficient decision making regarding compound prioritization.

Toxicogenomics and drug discovery: will new technologies help us produce better drugs?

Nature review/Drug Discovery 1:84-88(2002)

Acting on reports in the late 1980s that most drug candidates fail in development, pharmaceutical discovery programmes responded by devising ways to increase the number of chemicals in the pipeline. With discovery now driven primarily by chemistry and high-throughput screening, the biological effects and, in particular, the toxicity of new compounds are largely not appreciated until a compound enters development. Arguably, this paradigm has produced more failures rather than delivering more successes — with more chemicals to examine, much less is known about any single agent before costly development studies are initiated. The emerging field of toxicogenomics is enabling us to ask detailed questions about drug effects very early on, thereby fundamentally changing our approach to drug discovery.

"To save time and resources, drug safety and efficacy would ideally be determined simultaneously..."

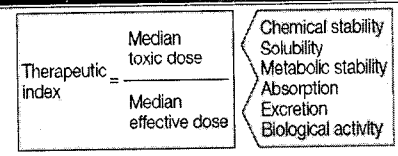


Figure 1 | **Therapeutic index.** The therapeutic index is a numerical measure of both the beneficial effect and relative safety of a new drug. Both efficacy and toxicity depend on several pharmacokinetic and biological parameters.

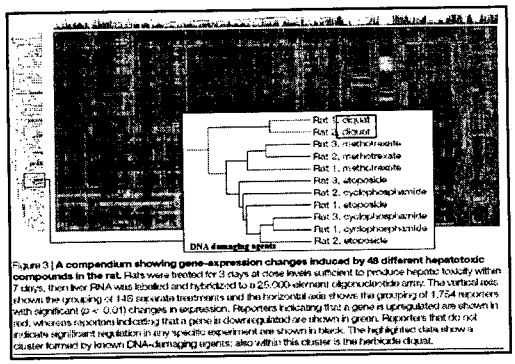


Figure 3 | **A compendium showing gene-expression changes induced by 48 different hepatotoxic compounds in the rat.** Rats were treated for 3 days at dose levels sufficient to produce hepatic toxicity within 7 days, then liver RNA was labelled and hybridized to a 25,000-element oligonucleotide array. The vertical axis shows the grouping of 145 separate treatments and the horizontal axis shows the grouping of 1,754 reporters with significant ($p < 0.01$) changes in expression. Reporters indicating that a gene is upregulated are shown in red, whereas reporters indicating that a gene is downregulated are shown in green. Reporters that do not indicate significant regulation in any specific experiment are shown in black. The highlighted data show a cluster formed by known DNA-damaging agents; also within this cluster is the herbicide diquat.

■RNA transcription Analysis
-DNA chip analysis can generate data relevant for understanding both the efficacy and the safety of a compound.

Toxicogenomics: a new revolution in drug safety

Drug discovery Today 7(13):728-736(2002)

New drugs are screened for adverse reactions using a laborious, costly process and still some promising therapeutics are withdrawn from the marketplace because of unforeseen human toxicity. Novel higher-throughput methods in toxicology need to be developed. These new approaches should provide more insight into potential human toxicity than current methods. Toxicogenomics, the examination of changes in gene expression following exposure to a toxicant, offers the potential to identify a human toxicant earlier in drug development and to detect human-specific toxicants that cause no adverse reaction in rats.

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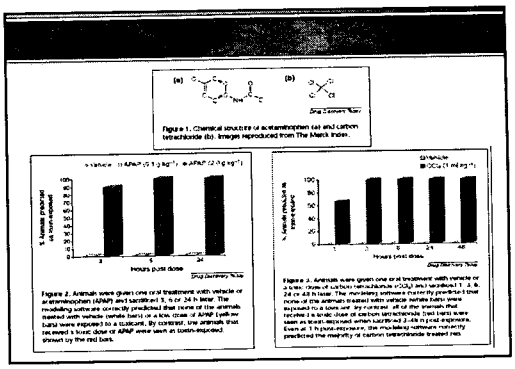

Toxicogenomics detection of a human-specific toxicant

Table 2. Comparisons of acetylcholinesterase inhibitors

Drug	Inhibitory action [62] Acetylcholinesterase	Butyrylcholinesterase	Hepatotoxicity Rats	Humans
Tacrine	Yes	Yes	No	Yes
Donepezil	Yes	No	No	No
Physostigmine	Yes	Yes	No	No

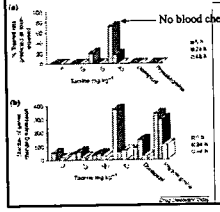

Figure 4. Animals were dosed orally once with vehicle, tacrine, donepezil or physostigmine and then sacrificed 6, 24 or 48 h later. (a) The percentage of animals identified, on the basis of ToxExpress™ (Gene Logic, Gaithersburg, MD, USA) modeling software, as exposed to toxicants or non-toxicants is illustrated. (b) The number of genes in each drug-treated group whose expression level changed in comparison to vehicle-treated rats is illustrated. Criteria used for this graph were genes exhibiting a twofold or greater change with statistical significant of $p < 0.01$. The illustration is not limited to genes showing predictive toxicity but portrays all genes present on the Affymetrix (Santa Clara, CA, USA) rat A microarray.

Gene expression profiling reveals multiple toxicity endpoints induced by hepatotoxicants

Mutation Research 549:147–168 (2004)

- Acetaminophen(APAP), methotrexate(MTX), methapyrilene(MP), furan and phenytoin(PHT) produced different hepatotoxic endpoints
- Current analyses demonstrate a good correlation between gene expression and hepatotoxic endpoints
- Subsets of genes related to necrosis, microvesicular, lipidosis, hepatocellular hypertrophy, bile duct hyperplasia and fibrosis were identified.
- Some gene expression changes preceded the occurrence of microscopic lesions, suggesting that expression profiling can be a more sensitive measure than histopathological examination in elucidating certain downstream hepatotoxicities.

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Different hepatotoxic endpoint

- Acetaminophen(APAP): centrilobular necrosis
- Methotrexate(MTX): atrophy, necrosis
- Methapyrilene(MP): periportal necrosis, bile duct hyperplasia
- Furan: hepatocellular carcinoma, bile duct hyperplasia, cholangiofibrosis, cholangiocarcinoma
- Phenytoin(PHT): hypertrophy

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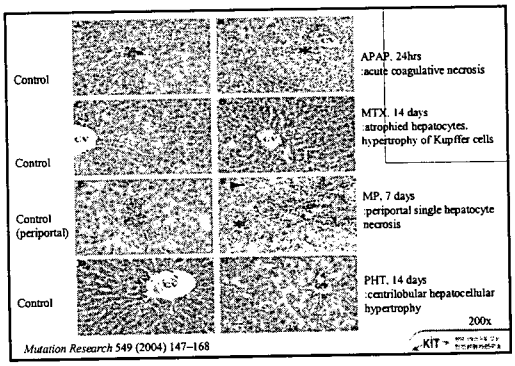
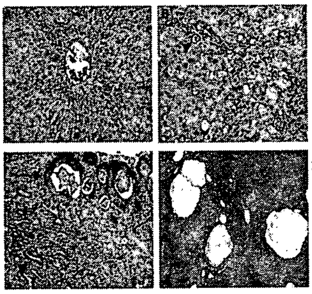


Control
: portal triad

Furan, 7 days
: degeneration
& necrosis



Furan, 14 days
: cholangiofibrosis,
vacuolated goblet
cells

:bile duct
hyperplasia
200x

Furan, 14 days
: abnormal large
cystic space in
the parenchyma

40x

Mutation Research 549 (2004) 147–168

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	APAP			MP			Furan				MTX			PHT
	6h	24h	3 day	24h	3 day	7 day	24h	3 day	7 day	14 day	24h	7 day	14 day	14 day
Hyperplasia (regenerative, hepatocellular)														
Minimal								1	1					
Mild									3					
Moderate										1				
Marked										3				
Cholangiofibrosis														
Minimal									1					
Mild										1				
Moderate										2				
Marked										1				
Centrilobular hepatocellular atrophy														
Minimal													1	
Mild													1	
Kupffer cell hypertrophy														
Minimal													1	
Centrilobular hepatocellular hypertrophy														
Moderate														4

Cholangio—

Note: All the hepatic lesions were drug-related and did not occur in the control livers, with the exception of one control animal in the 7 day control group of the furan study. This control exhibited mild vacuolation. The severity of lesions were graded as minimal, mild, moderate, and marked.

Mutation Research 549 (2004) 147–168

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Classification of significant gene expression changes induced by APAP, MP, furan, MTX, and phenytoin

Function categories gene name	Accession no.	APAP	MP	Furan	MTX	PHT
Inflammation						
Alpha-1 acid glycoprotein	AI022162	2.11	−0.63	1.41	3.25	
Alpha-fibrinogen	AA925421		−2.22	−1.06	3.16	
C-reactive protein	M83178	−1.08	−1.23	−2.63	1.05	
Cystatin C	X16957		0.84	0.91		
Beta-ATPase	Y11835				1.05	
IkB-a	X63594			1.54		
Integrin beta1	U12309	0.87	0.97	1.69		
Integrin beta-4	U60096			0.66		
Inter-alpha-inhibitor H4 heavy chain (Itih4)	AA901379		−0.86	−2.15	2.18	
Interferon gamma	AF010466			0.73	−2.13	
Interleukin-1 beta	M98820			0.80		
Interleukin-10	L02926			0.65		
Interleukin-6	M26744			0.58	−1.11	
Macrophage inflammatory protein-1 alpha	U22414			1.12		
Macrophage inflammatory protein-2 alpha	U45965	1.39	0.95	0.95		
MHC class I antigen RT1.A(l) alpha-chain	AA924962			0.67		
Monocyte chemotactic protein receptor (CCR2)	U77349			0.99		
Mx1 protein	NM_017028		1.02			
Osteopontin	AA964431			1.19		
Suppressor of cytokine signaling 3	AF075383				1.32	
Urokinase plasminogen activator receptor	AF007789	0.90				
Vascular cell adhesion molecule 1 (VCAM-1)	M84488			0.93		
Alpha-1-inhibitor III	J03552	−1.40	−1.68	−3.40	−2.46	
Alpha-1-antitrypsin	M32247			−0.95		
Alpha-1 microglobulin/bikunin precursor (Ambp)	AI043784		−0.94	−2.04		
C4b-binding protein	NM_012516		−1.41	−1.68		
Ceruloplasmin	L33869		−1.06	−1.69		
Complement component C3	X52477			−1.87		
Complement factor I (CFI)	AA900791			−0.63		

염증관련

Macrophage 관련

Inflammation, which usually accompanies tissue necrosis, was observed in livers treated with APAP, MP and furan. APAP and MP treatment induced mixed inflammatory cell infiltrates and mononuclear cell infiltrates respectively, whereas furan treatment produced mild to moderate hepatitis. Consistent with these histopathologic findings, acute phase proteins (alpha-1 acid glycoprotein, alpha-1-inhibitor III, and C-reactive protein), were differentially regulated in response to APAP, MP and furan treatments

Gene expression profiling can be used to…

1) Distinguish different hepatotoxic endpoints
2) Predict the development of toxic endpoints
3) Develop hypothesis regarding mechanisms of toxicity

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Biomarkers

Biological Marker (Biomarker) – A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic

Biomarker Definitions Working Group - 1998

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Automated learning of structure–activity relationships (SARs) from chemical and biological data

Application of SAR model to predict biological activities of untested compounds

EHP 108:1029–1033 (2000).

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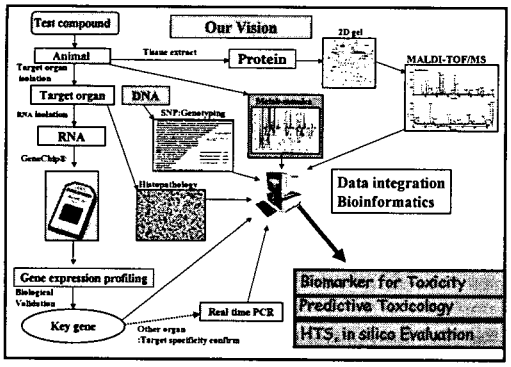
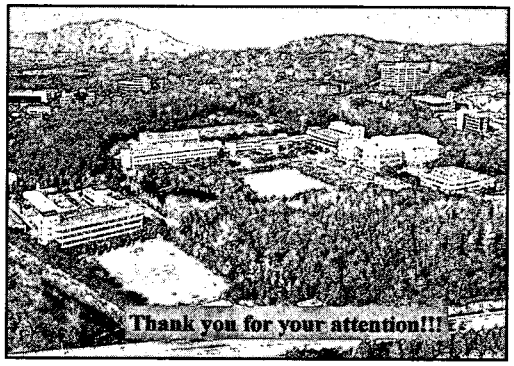

Test compound
Our Vision
Animal
Tissue extract
Protein
2D gel
MALDI-TOF/MS
Target organ isolation
Target organ
DNA
SNP:Genotyping
RNA Isolation
RNA
GeneChip®
Histopathology
Data integration
Bioinformatics
Gene expression profiling
Biological Validation
Key gene
Real time PCR
Other organ
:Target specificity confirm
Biomarker for Toxicity
Predictive Toxicology
HTS, in silico Evaluation


Thank you for your attention!!!