Lush Science Prize 2013

Background Paper

1 Executive Summary

1.1 Building on the work of the 2012 Background Paper

The Lush Science Prize is designed to reward 'outstanding contributions' to 21st Century Toxicology Research. In the 2012 Background Paper for the Lush Science Prize it was proposed that, in order to have a rational set of criteria to identify possible winners from what was potentially a very wide area, there was a need to focus in on a specific type of research to have an impact. Because of the Lush Prize's wider goal to encourage a breakthrough event (with a Black Box Prize) around a 'proof of concept toxicity pathway study', the focus chosen was on researchers working to understand 'toxicity pathways'.

This approach won approval from the Judges and the wider Prize community and therefore it has formed the basis for research for this 2013 Background Paper.

It should be noted that the wording on the nominations forms was changed slightly this year and this has been reproduced in Section 2 below.

1.2 Methodology

We looked at some key institutional developments in the area which included the OECD's Adverse Outcome Pathway Project, the Hamner Institute's work and the Human Toxome Project (Section 4 below). We also looked at the phenomenon of collaborative computer projects in this area (Section 5) and performed a brief literature review (Section 6).

However, in order to identify specifically 'toxicity pathway' breakthroughs, we focused, as last year, mainly on work published in peer-reviewed journals.

The journal search methodology we used is explained in Section 3 below and the 51 abstracts we selected for 'scoring' appear in Section 7. By this method two projects received the highest score and 21 projects received the next highest. Short descriptions of each of those receiving the lower score appear in an appendix at the end of this document.

1.3 1.3 Projects recommended for the shortlist

These are the two projects which received the highest scores.

In the event that insufficient nominations are received in the Science category this year, these projects could go forward to be considered by the judges, as happened last year. More details appear in the Conclusions.

1.3.1 Human embryonic stem cell-derived test systems for developmental neurotoxicity: a transcriptomics approach

Krug AK, Kolde R, Gaspar JA, Rempel E, Balmer NV, Meganathan K, Vojnits K, Baquié M, Waldmann T, Ensenat-Waser R, Jagtap S, Evans RM, Julien S, Peterson H, Zagoura D, Kadereit S, Gerhard D, Sotiriadou I, Heke M, Natarajan K, Henry M, Winkler J, Marchan R, Stoppini L, Bosgra S, Westerhout J, Verwei M, Vilo J, Kortenkamp A, Hescheler J, Hothorn L, Bremer S, van Thriel C, Krause KH, Hengstler JG, Rahnenführer J, Leist M, Sachinidis A.

Department of Biology, University of Konstanz, 78457 Constance, Germany.

"Developmental neurotoxicity (DNT) and many forms of reproductive toxicity (RT) often manifest themselves in functional deficits that are not necessarily based on cell death, but rather on minor changes relating to cell differentiation or communication. The fields of DNT/RT would greatly benefit from *in vitro* tests that allow the identification of toxicant-induced changes of the cellular proteostasis, or of its underlying transcriptome network. Therefore, the 'human embryonic stem cell (hESC)-derived novel alternative test systems (ESNATS)' European commission research project established RT tests based on defined differentiation protocols of hESC and their progeny. In conclusion, the ESNATS assay battery allows classification of human DNT/RT toxicants on the basis of their transcriptome profiles."

1.3.2 Profiling Environmental Chemicals for Activity in the Antioxidant Response Element Signaling Pathway Using a High Throughput Screening Approach

Sunita J. Shukla, Ruili Huang, Steven O. Simmons, Raymond R. Tice, Kristine L. Witt, Danielle VanLeer, Ram Ramabhadran, Christopher P. Austin, Menghang Xia

NIH Chemical Genomics Center USA, National Institutes of Health, Department of Health and Human Services, Rockville, Maryland, USA, U.S. Environmental Protection Agency, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, USA

"Oxidative stress has been implicated in the pathogenesis of a variety of diseases ranging from cancer to neurodegeneration, highlighting the need to identify chemicals that can induce this effect. The antioxidant response element (ARE) signaling pathway plays an important role in the amelioration of oxidative stress. Thus, assays that detect the up-regulation of this pathway could be useful for identifying chemicals that induce oxidative stress.

We used cell-based reporter methods and informatics tools to efficiently screen a large collection of environmental chemicals and identify compounds that induce oxidative stress. We utilized two cell-based ARE assay reporters, β-lactamase and luciferase, to screen a U.S. National Toxicology Program 1,408-compound library (NTP 1408, which contains 1,340 unique compounds) for their ability to

induce oxidative stress in HepG2 cells using quantitative high throughput screening (qHTS).

Our results support the robustness of using two different cell-based approaches for identifying compounds that induce ARE signaling. Together, these methods are useful for prioritizing chemicals for further in-depth mechanism-based toxicity testing."

1.4 Other Conclusions

At Section 5 below we note how the way that the Science Prize research, with its current focus on 'toxicity pathway breakthroughs', will always overlook some equally, if not more, important collaborative meta-initiatives (such as the Human Toxome Project) and some purely computational contributions. Some of these, it should be noted, are already very substantially funded.

It may be that one year, the Prize could choose to focus on and invite applications from, for example, purely computational projects in order to compensate for this.

2 Background

The Lush Prize website science prize nominations form text has been updated slightly from last year and currently reads as follows

Science

21st Century Toxicology Research

21st Century Toxicology is a new approach to safety testing which is exciting regulators, toxicologists, campaigners and companies around the world. It has become possible because of advances in biology, genetics, computer science and robotics.

21st-Century Toxicology focuses on human 'toxicity pathways', the sequences of molecular changes within the body's cells that follow exposure to a toxic chemical. As these molecular pathways are elucidated for different groups of chemicals and different toxic effects, computer technology will help identify the key steps that can then be used to design non-animal safety tests.

Many of these new tests will be done robotically, providing more cost-effective chemical assessment and helping to clear the backlog of untested substances. They offer better relevance to humans (rather than using mice, rats and rabbits), and will explain the underlying causes of toxicity. Unlike animal methods, the new tests will help predict human variability and differential effects on embryos, children and adults. And as the superior scientific basis of the new approach is recognised, outdated animal tests will be replaced.
For more information on this subject see:

Humane Society International (video)

National Academy of Sciences Introduction (3pp pdf)

Human Toxicology Project Document (2pp pdf)

US National Academy of Sciences (Book 196pp)

Anyone can nominate individuals, research teams or institutions for work conducted on relevant toxicity pathways. Applicants can also nominate themselves. Outstanding research producing an effective non-animal safety test based on an approach other than toxicity pathways, where none existed before, may also be considered. You can nominate an individual, team or institution below.

3 Methodology

In 2012, two recent major conferences on alternatives to animal testing had published a list of submitted papers:

- the 8th World Congress on Alternatives and Animal Use in the Life Sciences (WC8 at Montreal); and
- the European Society for Alternatives to Animal Testing (EUSAAT 14 at Linz).

The 2012 Background Paper was therefore able to explore the 381 research papers submitted in order to identify potential prize winning projects. For this 2013 Paper, a method using conference papers was not possible as these or similar events were not taking place this time round.

After a review of key institutions and publications (Section 4 and 6 below) we therefore decided to look at 'toxicity pathway' research published in peer-reviewed journals during 2012/13. In the 2012 Background Paper we identified how “an exploration of the journal aggregating website sciencedirect.com revealed that although there were only 21 journals with 'toxicology' in the title, there were 1078 with 'biochemistry, genetics and molecular biology' in the title. In order to reduce the research area to a manageable size, we reviewed only five key sources for new research published in the 2012/13 period:

Two directly relevant journals

- ALTEX Journals - looking at abstracts or citations (4 found)
- Toxicology In Vitro Abstracts – looking at abstracts (15 found)

One key annual review of progress in the area of 21st century toxicology research

- AXLR8 Progress Report 2012 (7 found)

Two broader electronic searches for 'toxicity pathway' (2012/13) among the wide range of journals searchable at:

- Google Scholar (6 selected)
- Terkko – Helsinki University Medical Library Search Engine (19 selected)

We then scored each abstract according to the method used in the 2012 Science Prize Background Paper as follows:

Does it appear to be reporting a new pathway discovery?	Score 3
If it is working with apparently previously understood pathway research, does it bring new knowledge or tools?	Score 2
Does it stand out in any other way?	Score 1

Each of the 51 abstracts that were initially identified appears in Section 7 below, along with their scoring. There were 21 projects scoring three points and two scoring four. Short descriptive extracts from the abstracts scoring 3 or more appear in Appendix 1 below with links to the journal publisher in each case.

It should be noted that the researcher working on this paper is a journalist and not a toxicologist and that the high volume of publications in this area means that only cursory analyses of each project was possible during this process.

4 Key Institutional Developments

4.1 OECD Adverse Outcome Pathways

One of the key elements of the Lush Prize is to focus attention on 21st Century Toxicology and its promise to learn about human toxicity pathways. It is therefore very significant to see that, since the last Background Paper, the OECD – not an organisation short of access to financial resources – has announced a project specifically in this area. Its website describes the project as follows:

> "In 2012, the OECD launched a new programme on the development of Adverse Outcome Pathways. An Adverse Outcome Pathway (AOP) is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organization that lead to an adverse health or ecotoxicological effect.

> AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning.
>
> Most of the AOP development and review is intended to take place via a web-based IT management tool ("AOP Knowledge Base") which is in development and is being tested until the end of 2013. More information will be made publicly available after the testing phase."[1]

There is also an open call for projects, and 26 were already listed on the website in August 2013. Most of these has 2013 start dates and will not have outputs yet. However they may be promising routes to identifying possible pathway breakthroughs in future years. We look at the web-based management tool in section 5.1 below.

4.2 The Hamner Institute and proof-of-concept toxicity pathway work

The US-based Hamner Institute is openly committed to working on the very proof-of-concept toxicity pathways that the Lush Black Box Prize is seeking to encourage.

"While all these research programmes are contributing to advancing risk assessment sciences with *in vitro* methods, proof-of-concept studies appear particularly important for assessing whether the specific recommendations of the NRC report are feasible based on current biological knowledge....At the Hamner, we have initiated programmes to develop case study approaches with six toxicity pathways: three receptor mediated pathways—aryl hydrocarbon (AhR), peroxisome proliferator-activated receptor-alpha (PPARα), and estrogen receptor (ER)—and three reactivity based pathways—DNA-damage, oxidative stress and mitochondrial toxicity. Our approaches are furthest along with PPARa and p53. The most extensive programme focuses on estrogen pathway signalling."[2]

Disappointingly, it appears that its use of 'limited' *in vivo* studies may make it ineligible for the Prize. "We are conducting similar *in vitro* studies with rat hepatocytes. Limited

in vivo studies in rats will focus on dose response of gene expression in rat livers after treatment with GW7647 and allow determination of the relevance of the dose-response seen *in vitro* in primary hepatocytes with that occurring *in vivo*."[3]

4.3 The Human Toxome Project

Also mentioned in the 2012 Background Paper, the Center for Alternatives to Animal Testing at Johns Hopkins University's programme to assess the human toxome and annotate the pathways associated with toxic responses continues. It has been funded with US $6 million over five years from the US National

[1] www.oecd.org/env/ehs/testing/molecularscreeningandtoxicogenomics.htm
[2] AXLR8 2012 Progress Report pp142-7
[3] AXLR8 2012 Progress Report pp145

Institutes of Health, plus extra support from the Environmental Protection Agency and the Food and Drug Administration.[4]

In this project, "studies in validated cell systems define pathways of toxicity (PoTs) in human cells *in vitro* in order to develop mode-of-action based mechanistic information for assessing risks posed by exposure to various substances. The eventual goal is to map all pathways of what is called 'the human toxome'. The ongoing project at Johns Hopkins focuses on estrogen pathway signalling in MCF-7 cells. Validated methods already exist for using these cells to assess estrogenicity. Using the reverse dosimetry approach developed jointly between the Hamner Institutes and staff at the US EPA National Center for Computational Toxicology (NCCT), concentrations that elicit cellular responses become the basis for estimating safe human exposure conditions (or exposures that pose a *de minimis* risk level)."[5]

They appear to visualise a similar collaborative web tool to those worked on by others (see section 5 below). "Beyond that, this project will develop a common, community-accessible framework and databases that will enable the toxicology community at large to comprehensively and cooperatively map the human toxome using integrated testing strategies that combine "omics" data with computational models."[6]

5 Collaborative computational systems

5.1 OECD Adverse Outcome Pathway Wiki

The announcement, in 2012, of the Adverse Outcome Pathway Wiki project by the OECD appears to be an important step towards mapping toxicity pathways in a common format. This was described on the JRC's website as follows:

> "An IT system to capture, manage and share Adverse Outcome Pathways - AOP Wiki - has been made available for testing to an initial user group comprising current contributors to the OECD's AOP Development Programme, including members of the OECD Extended Advisory Group on Molecular Screening and Toxicogenomics (EAG MST) and the WHO/IPCS Mode of Action project group.
>
> The Wiki leads AOP developers through the steps necessary to capture the scientific information needed to document and evaluate an AOP following recently published OECD guidance and provides an ideal collaboration space for international AOP-development project teams. A Wikipedia-like system of user-friendly tables, drop-down-boxes and built-in functionality for automatic cross-referencing between related pages makes the tool extremely easy to use. A series of webinars are planned to familiarise the first user group with the process of entering information into the AOP Wiki and keeping their AOPs updated.

[4] http://www.nature.com/news/big-biology-the-omes-puzzle-1.12484
[5] AXLR8 2012 Progress Report pp147
[6] http://humantoxome.com homepage viewed August 2013

The beta-testing phase will last until the end of 2013, when the next development cycle will start. The system is expected to become freely available (read access) to the international scientific community by January 2014."[7]

5.2 Effectopedia: The Online Encyclopaedia and Graphical Editor for AOPs

To some extent the OECD project appears to be working in the same area as a project of the International QSAR Foundation (which has received OECD funding). The effectopedia project is, however, already active and is described in the following poster, presented at the EPAA annual conference by Gilly Stoddart of PETA.[8]

> "The 21st Century shift to more prospective hazard identification and hypothesis generation requires greater strategic application of systems biology, QSAR and archived toxicological data in the form of adverse outcome pathways (AOPs). AOPs describe the causal linkages among biological responses to chemicals over time. The complexity of integrating science can be a barrier to progress in terms of the toxicity pathways and networks involved as well as the need to organize knowledge from many disciplines.
>
> Effectopedia is an open-knowledge aggregation and collaboration tool for delineating AOPs in an encyclopedic and predictive manner. It includes discrete cause-effect studies and critical reviews that are relevant to toxicology. To achieve human and machine interpretability, Effectopedia uses an ontology-enhanced, natural language interface that offers clarifying questions and special tags to define the semantic knowledge while preserving the natural language description of the AOP's elements. Effectopedia serves as a graphical editor to delineate causal linkages at any level of biological organization and species. It creates a common organizational space that (1) helps experts identify gaps in knowledge of causal linkages of biological responses and (2) acts as a web-based
>
> conference room for dialogue and synthesis by experts with interest in specific AOPs. Effectopedia's live documents are instantly open for discussions and feedback, whilst giving credit to original authors and reviewers. New contributions are immediately distributed to interested parties. Uncoupling the contribution and review processes permits organizations to define their own seals of approval and associate them with special interest pathways without slowing down the Wiki-inspired contributions."

It should be noted that similar – though not necessarily collaborative – databases covering similar areas also exist. The Max Planck Institute for Molecular Genetics, for example, manages the ConsensusPathDB resource (cpdb.molgen.mpg.de), a meta-database that integrates the content of 22 different interaction databases and that comprises 2,144 predefined human pathways of which 1,695 had more than five members and were used for pathway response analysis.[9]

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[7] http://ihcp.jrc.ec.europa.eu/our_activities/alt-animal-testing-safety-assessment-chemicals/improved_safety_assessment_chemicals/first-release-of-aop-wiki/

[8] http://ec.europa.eu/enterprise/epaa/3_events/ann-conf-2012/poster-book.pdf

[9] AXLR8 2012 Progress Report p177

As we mentioned in the 2012 Background Paper, the way the Science Prize currently focuses on 'toxicity pathway' breakthroughs means that some of these equally valuable collaborative meta-initiatives and computational contributions will be overlooked. It may be that one year, the Prize could choose to focus on and invite applications from purely computational projects in order to compensate for this.

6 Brief Literature Review

A wider literature review of key 21st Century Toxicology texts took place in the 2012 Science Background Paper and has not been repeated here. This year we began by absorbing the AXLR8 2012 Progress Report which is designed to report annually on alternative testing strategies. It also describes in detail some of the very substantially funded EU projects such as SEURAT 1 (systematic dose replacements) which are still under way. It has been used as a key resource throughout this Background Paper and is not otherwise discussed separately here.

We have also systematically reviewed the ALTEX journal which has reproduced a number of discursive analyses of current developments in 21st Century Toxicology, but the following 'roadmap' review from Basketter et al (ALTEX 29, 1/12 3) stands out as a useful summary of progress in five key areas.

The main conclusions and recommendations of the roadmap were summarised as follows:

> "1. Toxicokinetics
>
> – Represents a necessary complement to all in vitro approaches to allow QIVIVE.
>
> – Need for "in vitro kinetics" of chemicals in the experimental systems with the goal of producing proper kinetic parameters for QIVIVE.
>
> – In silico approaches need to be further optimised.
>
> – Need for more comprehensive data collections, especially in vitro data from barrier models.
>
> – Problems mainly in the fields of bioavailability and urinary excretion.
>
> – Achievable with reasonable investment.
>
> 2. Sensitisation
>
> – Reasonably good animal model (LLNA) capable of generating potency and dose response information.

– Multiple in vitro assays available but unclear which test methods provide potency information.

– The need to build mechanistic understanding to enable data integration for potency determination for hazard characterisation & risk assessment remains an important in vitro challenge.

3. Repeated dose testing

– Tox-21c approaches based on PoT represent the key perspective; need to focus on defining levels that cause. -Adverse effects rather than just hazard identification.

– Need for data sharing from industry.

– Need for models for PoT identification (e.g., stem cells).

– Need for co-cultures, 3D models, and long-term models.

– Human disease knowledge and known toxicants must be exploited.

– Increased focus on modelling of inflammatory/immunological damage.

4. Carcinogenicity

– Possible abolition of current test via an objective assessment with tools of evidence-based toxicology (EBT).

– Important ongoing work to optimise genetic toxicity battery.

– Further evaluation of cell transformation assay required.

– ITS including non-genotoxic modes of action should be developed.

– Tox-21c approaches based on PoT (including metabolomics) represent a key opportunity.

5. Reproductive Toxicity

– Analysis of current animal tests by EBT approaches.

– Validation of (human) embryonic stem cell test variants.

– Validation of zebrafish egg test for teratogenicity.

– Extension of ITS approaches, extending the approach of ReProTect

– Extension of the ToxCast program currently pioneering PoT-based assessments

– Tox-21c approaches based on PoT, especially mapping the PoT for reproductive toxicity for a Human Toxome database."[10]

7 Searching for Toxicity Pathway Journal Abstracts

This section contains a number of tables which display in the first column an abstract title and in the second column a 'score' using the 2012 Background Paper methodology.

Does it appear to be reporting a new pathway discovery?	Score 3
If it is working with apparently previously understood pathway research, does it bring new knowledge or tools?	Score 2
Does it stand out in any other way?	Score 1

7.1 ALTEX

ALTEX 2012 and 2013 was searched both for its own published research and for news of other potentially useful publications or breakthroughs. The following four pieces of research stood out with their scores appearing in the final column.

Human embryonic stem cell-derived test systems for developmental neurotoxicity: a transcriptomics approach	3+1
A 3-dimensional human embryonic stem cell (hESC)-derived model to detect developmental neurotoxicity of nanoparticles	2
Perspectives on validation of high-throughput assays supporting 21st century toxicity testing	1
A 155-plex high-throughput *in vitro* coregulator binding assay for (anti-)estrogenicity testing evaluated with 23 reference compounds	3

7.2 Toxicology In Vitro Abstracts

We searched for 'pathway' in Toxicology In Vitro from August 2012 to present. Quite a few are chemicals being tested against known pathways.

Cytotoxic responses in BC3H1 myoblast cell lines exposed to 1-desulfoyessotoxin	2

[10] t4 Report A Roadmap for the Development of Alternative (Non-Animal) Methods for Systemic Toxicity Testing http://www.altex.ch/resources/altex_2012_1_003_092_Basketter_full_final.pdf

Cooperation of bisphenol A and leptin in inhibition of caspase-3 expression and activity in OVCAR-3 ovarian cancer cells	2
Assessing dose-dependent differences in DNA-damage, p53 response and genotoxicity for quercetin and curcumin	2
Time course study of Aβ formation and neurite outgrowth disruption in differentiated human neuroblastoma cells exposed to H_2O_2: Protective role of autophagy	2
Ethanol-induced apoptosis in human liver adenocarcinoma cells (SK-Hep1): Fas- and mitochondria-mediated pathways and interaction with MAPK signaling system	3?
Induction of HepG2 cell apoptosis by Irgarol 1051 through mitochondrial dysfunction and oxidative stresses	2
Melamine activates NFκB/COX-2/PGE2 pathway and increases NADPH oxidase–dependent ROS production in macrophages and human embryonic kidney cells	2
Applicability of a keratinocyte gene signature to predict skin sensitizing potential	3
Silver nanoparticles induce toxicity in A549 cells via ROS-dependent and ROS-independent pathways	3
Sodium arsenite induces cyclooxygenase-2 expression in human uroepithelial cells through MAPK pathway activation and reactive oxygen species induction	3
Cerium oxide nanoparticles induce cytotoxicity in human hepatoma SMMC-7721 cells via oxidative stress and the activation of MAPK signaling pathways	2
Methylglyoxal induces DNA crosslinks in ECV304 cells via a reactive oxygen species-independent protein carbonylation pathway	2
T-2 toxin enhances catabolic activity of hypertrophic chondrocytes through ROS-NF-κB-HIF-2α pathway	2
Critical roles of Rho-associated kinase in membrane blebbing and mitochondrial pathway of apoptosis caused by 1-butanol	2
Impacts of low doses of pesticide mixtures on liver cell defence systems	2

7.3 AXLR8 Citations from 2012

A Computational Model Predicting Disruption of Blood Vessel Development	2+1
Putting the parts together: combining *in vitro* methods to test for skin sensitizing potentials	2+1
Allergic contact dermatitis: a commentary on the relationship between T lymphocytes and skin sensitising potency.	2
An *in vitro* method for detecting chemical sensitization using human reconstructed skin models and its applicability to cosmetic, pharmaceutical, and medical device safety testing.	3
Profiling Environmental Chemicals for Activity in the Antioxidant Response Element Signaling Pathway Using a High Throughput Screening Approach	3+1
Focus on Stem Cells as Sources of Human Target Cells for *In Vitro* Research and Testing	1
Development of new structural alerts suitable for chemical category formation for assigning covalent and non-covalent mechanisms relevant to DNA binding	

7.4 Google Scholar

Google scholar produced 251 results for a search of 'toxicity pathway' between 2012-2013 (200 were scanned). Ignoring animal tests and disease-related research (e.g. cancer, Alzheimer's) the following abstracts were scored.

Quantitative High-Throughput Screening for Chemical Toxicity in a Population-Based *In Vitro* Model	1
Predictive toxicology using systemic biology and liver microfluidic "on chip" approaches: Application to acetaminophen injury	2
Mechanisms of toxicity of triphenyltin chloride (TPTC) determined by a live cell reporter array	3
Effects of usnic acid exposure on human hepatoblastoma HepG2 cells in culture	2
A systematic study of mitochondrial toxicity of environmental chemicals using quantitative high throughput screening	3

Reactivity of Chemical Sensitizers Toward Amino Acids *In Cellulo* Plays a Role in the Activation of the Nrf2-ARE Pathway in Human Monocyte Dendritic Cells and the THP-1 Cell Line	3

7.5 Terkko – Helsinki University Medical Library

Terkko has a particularly useful 'feed navigator' which indexes journals from Pub med, Elsevier, Singer, Science Direct, Oxford Journals, ALTEX etc.

In August 2013 we searched LifeSciencesJournals – Pharmacology, toxicology and pharmaceutics for “toxicity pathway”

http://www.terkko.helsinki.fi/feednavigator

With around 450 results, we eliminated by reading the titles only: clearly cancer or disease related studies, some nanotech research, some heavy metals toxicity research and some pesticides research.

It is clear that much toxicity pathway research is happening in cancer research communities which are looking to ameliorate drug side effects as well as looking to create toxic damage to cancer cells. Reading the remaining abstracts, we then eliminated those using animal testing in the broader research which left the following 19 abstracts.

The proteasome cap RPT5/Rpt5p subunit prevents aggregation of unfolded ricin A chain	2
Use and validation of HT/HC assays to support 21st century toxicity evaluations	1
Neuregulin-1β regulates tyrosine kinase receptor expression in cultured dorsal root ganglion neurons with excitotoxicity induced by glutamate	3
Modeling drug- and chemical-induced hepatotoxicity with systems biology approaches.	3
Gene and protein responses of human lung tissue explants exposed to ambient particulate matter of different sizes.	3
Unraveling amyloid toxicity pathway in NIH3T3 cells by a combined proteomic and 1H-NMR metabonomic approach	3
Differential Mitochondrial Toxicity Screening and Multi-Parametric Data Analysis	2

Discovery of Inhibitors of Microglial Neurotoxicity Acting Through Multiple Mechanisms Using a Stem-Cell-Based Phenotypic Assay	3
Metabolomic Response of Human Embryonic Stem Cell-Derived Germ-like Cells After Exposure to Steroid Hormones	2
Concentration-dependent induction of reactive oxygen species, cell cycle arrest and apoptosis in human liver cells after nickel nanoparticles exposure	2
Protein kinase CK2-dependent phosphorylation of the human Regulators of Calcineurin reveals a novel mechanism regulating the calcineurin-NFATc signaling pathway.	3
A signaling cascade mediated by ceramide, src and PDGFRβ coordinates the activation of the redox-sensitive neutral sphingomyelinase-2 and sphingosine kinase-1	3
Toxicometabolomics	1
Evaluation of a human neurite growth assay as specific screen for developmental neurotoxicants.	2
Perspectives for integrating human and environmental risk assessment and synergies with socio-economic analysis.	1
Global Gene Expression Profiling Reveals Functional Importance of Sirt2 in Endothelial Cells under Oxidative Stress.	3
Compound selection for *in vitro* modeling of developmental neurotoxicity.	1
A 3-dimensional human embryonic stem cell (hESC)-derived model to detect developmental neurotoxicity of nanoparticles.	2
Mechanism of the toxicity induced by natural humic acid on human vascular endothelial cells	2

8 Conclusions

Using the methodology described in Section 7 above, there were 21 projects scoring three and two scoring four. Short descriptive extracts from the abstracts for each of these scoring 3 or more appear in Appendix 1 below with links to the journal publisher in each case.

The two abstracts which scored highest (4) are reproduced in full here as follows. It will be worth considering whether they should be added to the 2013 Prize Shortlist.

8.1 Human embryonic stem cell-derived test systems for developmental neurotoxicity: a transcriptomics approach

Archives of Toxicology January 2013, Volume 87, Issue 1, pp 123-143

Krug AK, Kolde R, Gaspar JA, Rempel E, Balmer NV, Meganathan K, Vojnits K, Baquié M, Waldmann T, Ensenat-Waser R, Jagtap S, Evans RM, Julien S, Peterson H, Zagoura D, Kadereit S, Gerhard D, Sotiriadou I, Heke M, Natarajan K, Henry M, Winkler J, Marchan R, Stoppini L, Bosgra S, Westerhout J, Verwei M, Vilo J, Kortenkamp A, Hescheler J, Hothorn L, Bremer S, van Thriel C, Krause KH, Hengstler JG, Rahnenführer J, Leist M, Sachinidis A.

Developmental neurotoxicity (DNT) and many forms of reproductive toxicity (RT) often manifest themselves in functional deficits that are not necessarily based on cell death, but rather on minor changes relating to cell differentiation or communication. The fields of DNT/RT would greatly benefit from *in vitro* tests that allow the identification of toxicant-induced changes of the cellular proteostasis, or of its underlying transcriptome network. Therefore, the 'human embryonic stem cell (hESC)-derived novel alternative test systems (ESNATS)' European commission research project established RT tests based on defined differentiation protocols of hESC and their progeny. Valproic acid (VPA) and methylmercury (MeHg) were used as positive control compounds to address the following fundamental questions: (1) Does transcriptome analysis allow discrimination of the two compounds? (2) How does analysis of enriched transcription factor binding sites (TFBS) and of individual probe sets (PS) distinguish between test systems? (3) Can batch effects be controlled? (4) How many DNA microarrays are needed? (5) Is the highest non-cytotoxic concentration optimal and relevant for the study of transcriptome changes? VPA triggered vast transcriptional changes, whereas MeHg altered fewer transcripts. To attenuate batch effects, analysis has been focused on the 500 PS with highest variability. The test systems differed significantly in their responses (<20 % overlap). Moreover, within one test system, little overlap between the PS changed by the two compounds has been observed. However, using TFBS enrichment, a relatively large 'common response' to VPA and MeHg could be distinguished from 'compound-specific' responses. In conclusion, the ESNATS assay battery allows classification of human DNT/RT toxicants on the basis of their transcriptome profiles.

The full article is freely available on the web at:

http://link.springer.com/article/10.1007%2Fs00204-012-0967-3#page-1

8.2 Profiling Environmental Chemicals for Activity in the Antioxidant Response Element Signaling Pathway Using a High Throughput Screening Approach

Environ. Health Perspect. 120:1150-1156 (2012).
http://dx.DOI.org/10.1289/ehp.1104709

Sunita J. Shukla[1], Ruili Huang[1], Steven O. Simmons[2], Raymond R. Tice[3], Kristine L. Witt[3], Danielle VanLeer[1], Ram Ramabhadran[2], Christopher P. Austin[1], Menghang Xia[1]

[1]NIH Chemical Genomics Center, National Institutes of Health, Department of Health and Human Services, Rockville, Maryland, USA; [2]U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA; [3]Division of the National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, North Carolina, USA

BACKGROUND:

Oxidative stress has been implicated in the pathogenesis of a variety of diseases ranging from cancer to neurodegeneration, highlighting the need to identify chemicals that can induce this effect. The antioxidant response element (ARE) signaling pathway plays an important role in the amelioration of oxidative stress. Thus, assays that detect the up-regulation of this pathway could be useful for identifying chemicals that induce oxidative stress.

OBJECTIVES:

We used cell-based reporter methods and informatics tools to efficiently screen a large collection of environmental chemicals and identify compounds that induce oxidative stress.

METHODS:

We utilized two cell-based ARE assay reporters, β-lactamase and luciferase, to screen a U.S. National Toxicology Program 1,408-compound library (NTP 1408, which contains 1,340 unique compounds) for their ability to induce oxidative stress in HepG2 cells using quantitative high throughput screening (qHTS).

RESULTS:

Roughly 3% (34 of 1,340) of the unique compounds demonstrated activity across both cell-based assays. Based on biological activity and structure-activity relationship profiles, we selected 50 compounds for retesting in the two ARE assays and in an additional follow-up assay that employed a mutated ARE linked to β-lactamase. Using this strategy, we identified 30 compounds that demonstrated activity in the ARE-bla and ARE-luc assays and were able to determine structural features conferring compound activity across assays.

CONCLUSIONS:

Our results support the robustness of using two different cell-based approaches for identifying compounds that induce ARE signaling. Together, these methods are useful for prioritizing chemicals for further in-depth mechanism-based toxicity testing.

www.ncbi.nlm.nih.gov/pubmed/22551509

Appendix I

Extracts from each abstract scoring 3 or more have been reproduced below to give an indication of the nature of the research. A link at the bottom of each piece connects to a full version of each abstract available on the web. Where multiple institutions have been involved in the research, sometimes just the first-named appears in the credits for reasons of space.

- **ALTEX**

Archives of Toxicology January 2013, Volume 87, Issue 1, pp 123-143,

Human embryonic stem cell-derived test systems for developmental neurotoxicity: a transcriptomics approach

Krug AK, Kolde R, Gaspar JA, Rempel E, Balmer NV, Meganathan K, Vojnits K, Baquié M, Waldmann T, Ensenat-Waser R, Jagtap S, Evans RM, Julien S, Peterson H, Zagoura D, Kadereit S, Gerhard D, Sotiriadou I, Heke M, Natarajan K, Henry M, Winkler J, Marchan R, Stoppini L, Bosgra S, Westerhout J, Verwei M, Vilo J, Kortenkamp A, Hescheler J, Hothorn L, Bremer S, van Thriel C, Krause KH, Hengstler JG, Rahnenführer J, Leist M, Sachinidis A.

Department of Biology, University of Konstanz, 78457 Constance, Germany.

> Developmental neurotoxicity (DNT) and many forms of reproductive toxicity (RT) often manifest themselves in functional deficits that are not necessarily based on cell death, but rather on minor changes relating to cell differentiation or communication. The fields of DNT/RT would greatly benefit from *in vitro* tests that allow the identification of toxicant-induced changes of the cellular proteostasis, or of its underlying transcriptome network. Therefore, the 'human embryonic stem cell (hESC)-derived novel alternative test systems (ESNATS)' European commission research project established RT tests based on defined differentiation protocols of hESC and their progeny. In conclusion, the ESNATS assay battery allows classification of human DNT/RT toxicants on the basis of their transcriptome profiles.

http://www.ncbi.nlm.nih.gov/pubmed/23179753

ALTEX. 2013;30(2):145-57.

A 155-plex high-throughput *in vitro* coregulator binding assay for (anti-) estrogenicity testing evaluated with 23 reference compounds

Si Wang[1,2], René Houtman[3], Diana Melchers[3], Jac Aarts[1,2], Ad Peijnenburg[2], Rinie van Beuningen[3], Ivonne Rietjens[1], and Toine F. Bovee[2]

[1]Division of Toxicology, Wageningen University and Research Centre, Wageningen, The Netherlands; [2]Toxicology & Bioassays, RIKILT– Institute of Food Safety, Wageningen University and Research Centre, Wageningen, The Netherlands; [3]PamGene International B.V.'s-Hertogenbosch, The Netherlands

To further develop an integrated *in vitro* testing strategy for replacement of *in vivo* tests for (anti-)estrogenicity testing, the ligand-modulated interaction of coregulators with estrogen receptor α was assessed using a PamChip® plate. The relative estrogenic potencies determined, based on ERα binding to coregulator peptides in the presence of ligands on the PamChip® plate, were compared to the relative estrogenic potencies as determined in the *in vivo* uterotrophic assay. Moreover, this coregulator binding assay is able to distinguish ER agonists from ER antagonists: profiles of selective estrogen receptor modulators, such as tamoxifen, were distinct from those of pure ER agonists, such as dienestrol. Combination of this coregulator binding assay with other types of *in vitro* assays, e.g., reporter gene assays and the H295R steroidogenesis assay, will frame an *in vitro* test panel for screening and prioritization of chemicals, thereby contributing to the reduction and ultimately the replacement of animal testing for (anti-)estrogenic effects.

http://www.altex.ch/resources/raltex_2013_2_145_157_Wang4f1.pdf

- **Toxicology In Vitro**

Toxicology In Vitro Volume 27, Issue 6, September 2013, Pages 1820–1829

Ethanol-induced apoptosis in human liver adenocarcinoma cells (SK-Hep1): Fas- and mitochondria-mediated pathways and interaction with MAPK signaling system

Yuri Morio, Mayumi Tsuji, Manami Inagaki, Mai Nakagawa, Yuri Asaka, Hideto Oyamada, Kanji Furuya, Katsuji Oguchi

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For studying molecular mechanisms regulating the fate of ethanol-treated hepatocytes, involvement of Fas in ethanol-induced apoptosis was examined in human liver adenocarcinoma (SK-Hep1) cells in which the function of Fas-associated death domain (FADD) protein was knocked down by transfection. In FADD-knocked down cells, while ethanol-induced increase in generation of reactive oxygen species (ROS) was unaffected, apoptosis was significantly suppressed, demonstrating the involvement of Fas in ethanol-induced hepatocyte apoptosis more directly than in the past reports. We concluded that oxidative stress inflicted by reactive oxygen species (ROS) triggered Fas-mediated and mitochondria-mediated apoptotic pathways in ethanol-treated SK-Hep1 cells, and that p38 mitogen-activated protein kinase (MAPK) and JNK were promoting mitochondrial pathway, suggesting interaction between apoptosis and MAPK signaling systems.

www.sciencedirect.com/science/article/pii/S0887233313001355

Toxicology in Vitro Volume 27, Issue 1, February 2013, Pages 314–322

Applicability of a keratinocyte gene signature to predict skin sensitizing potential

Jochem W. van der Veen, Tessa E. Pronk, Henk van Loveren, Janine Ezendam

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There is a need to replace animal tests for the identification of skin sensitizers and currently many alternative assays are being developed that have very promising results. In this study a gene signature capable of very accurate identification of sensitizers was established in the HaCaT human keratinocyte cell line. This signature was evaluated in a separate study using six chemicals that are either local lymph node (LLNA) false-positive or false-negative chemicals in addition to nine sensitizers and four non-sensitizers. Similar studies do not apply these more difficult to classify chemicals, which show the true potential for human predictions of an assay. Although the gene signature has improved prediction accuracy compared to the LLNA, the misclassified compounds were comparable between the two assays. Gene profiling also showed a sensitizer specific response of the Nrf2-keap1 and Toll-like receptor signaling pathways. After exposure to non-sensitizing chemicals that induce either of the pathways the signature misclassified all Nrf2-inducers, while the Toll-like receptor ligands were correctly classified. In conclusion, we confirm that keratinocyte based prediction assays may provide essential information on the properties of compounds. Furthermore, chemical selection is critical for assessment of the performance of *in vitro* alternative assays.

www.sciencedirect.com/science/article/pii/S0887233312002305

Toxicology in Vitro Volume 27, Issue 1, February 2013, Pages 330–338

Silver nanoparticles induce toxicity in A549 cells via ROS-dependent and ROS-independent pathways

Porntipa Chairuangkitti, Somsong Lawanprasert, Sittiruk Roytrakul, Sasitorn Aueviriyavit, Duangkamol Phummiratch, Kornphimol Kulthong, Pithi Chanvorachote, Rawiwan Maniratanachote

Faculty of Pharmaceutical Sciences, Chulalongkorn University, 254 Phayathai Rd., Pathumwan, Bangkok 10330, Thailand

Silver nanoparticles (AgNPs) are incorporated into a large number of consumer and medical products. Several experiments have demonstrated that AgNPs can be toxic to the vital organs of humans and especially to the lung. The present study evaluated the *in vitro* mechanisms of AgNP (<100nm) toxicity in relationship to the generation of reactive oxygen species (ROS) in A549 cells. These observations

allow us to propose that the *in vitro* toxic effects of AgNPs on A549 cells are mediated via both ROS-dependent (cytotoxicity) and ROS-independent (cell cycle arrest) pathways.

www.sciencedirect.com/science/article/pii/S0887233312002287

Toxicology in Vitro Volume 27, Issue 3, April 2013, Pages 1043–1048

Sodium arsenite induces cyclooxygenase-2 expression in human uroepithelial cells through MAPK pathway activation and reactive oxygen species induction

Huihui Wang, Shuhua Xi, Yuanyuan Xu, Fei Wang, Yi Zheng, Bing Li, Xin Li, Quanmei Zheng, Guifan Sun

Department of Occupational and Environmental Health, School of Public Health, China Medical University, Shenyang, Liaoning, PR China

Arsenic can induce reactive oxygen species (ROS) leading to oxidative stress and carcinogenesis. Bladder is one of the major target organs of arsenic, and cyclooxygenase-2 (COX-2) may play an important role in arsenic-induced bladder cancer. However, the mechanism by which arsenic induces COX-2 in bladder cells remains unclear. This study aimed at investigating arsenic-mediated intracellular redox status and signaling cascades leading to COX-2 induction in human uroepithelial cells (SV-HUC-1). These data indicate that arsenite promotes an induction of ROS, which results in an induction of COX-2 expression through activation of the MAPK pathway.

www.sciencedirect.com/science/article/pii/S0887233313000131

- **AXLR8**

PLOS Computational Biology- Research Article Published: April 4, 2013

A Computational Model Predicting Disruption of Blood Vessel Development

Nicole Kleinstreuer, David Dix, Michael Rountree, Nancy Baker, Nisha Sipes, David Reif, Richard Spencer, Thomas Knudsen

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Vascular development is a complex process regulated by dynamic biological networks that vary in topology and state across different tissues and developmental stages. Simulating these interactions at a systems level requires sufficient biological detail about the relevant molecular pathways and associated cellular behaviors, and tractable computational models that offset mathematical and biological complexity. Here, we describe a novel multicellular agent-based model of vasculogenesis using

the CompuCell3D (http://www.compucell3d.org/) modeling environment supplemented with semi-automatic knowledgebase creation.

The model was shown to recapitulate stereotypical capillary plexus formation and structural emergence of non-coded cellular behaviors, such as a heterologous bridging phenomenon linking endothelial tip cells together during formation of polygonal endothelial cords. Molecular targets in the computational model were mapped to signatures of vascular disruption derived from *in vitro* chemical profiling using the EPA's ToxCast high-throughput screening (HTS) dataset. These findings support the utility of cell agent-based models for simulating a morphogenetic series of events and for the first time demonstrate the applicability of these models for predictive toxicology. This work was funded by the U.S. EPA.

www.ploscompbiol.org/article/info%3ADOI%2F10.1371%2Fjournal.pcbi.1002996

Regul. Toxicol. Pharmacol. 2012 Aug;63(3):489-504 (DOI: 10.1016/j.yrtph.2012.05.013. Epub 2012 Jun 1)

Putting the parts together: combining *in vitro* methods to test for skin sensitizing potentials.

Bauch C, Kolle SN, Ramirez T, Eltze T, Fabian E, Mehling A, Teubner W, van Ravenzwaay B, Landsiedel R.

BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany.

Allergic contact dermatitis is a common skin disease and is elicited by repeated skin contact with an allergen. The mechanisms that trigger skin sensitization are complex and various steps are involved. Therefore, a single *in vitro* method may not be able to accurately assess this endpoint. In this study, the predictivities of four *in vitro* assays, one *in chemico* and one *in silico* method addressing three different steps in the development of skin sensitization were assessed using 54 test substances of known sensitizing potential. The predictivity of single tests and combinations of these assays were compared. These data were used to develop an *in vitro* testing scheme and prediction model for the detection of skin sensitizers based on protein reactivity, activation of the Keap-1/Nrf2 signaling pathway and dendritic cell activation.

www.ncbi.nlm.nih.gov/pubmed/22659254

Cutaneous and Ocular Toxicology. 2012 Dec;31(4):292-305. (DOI: 10.3109/15569527.2012.667031. Epub 2012 Apr 12)

An *in vitro* method for detecting chemical sensitization using human reconstructed skin models and its applicability to cosmetic, pharmaceutical, and medical device safety testing.

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Chemical sensitization is a serious condition caused by small reactive molecules and is characterized by a delayed type hypersensitivity known as allergic contact dermatitis (ACD). Although most of the [non animal] research has focused on pure chemicals that possess reasonable solubility properties, it is important for any successful *in vitro* method to have the ability to test compounds with low aqueous solubility. The aim of this research was to demonstrate the functionality and applicability of the human reconstituted skin models (MatTek Epiderm(®) and SkinEthic RHE) as a test system for the evaluation of chemical sensitization and its potential use for medical device testing. In addition, the development of the human 3D skin model should allow the *in vitro* sensitization assay to be used for finished product testing in the personal care, cosmetics, and pharmaceutical industries. This approach combines solubility, chemical reactivity, cytotoxicity, and activation of the Nrf2/ARE expression pathway to identify and categorize chemical sensitizers.

The results demonstrated that both the MatTek and SkinEthic models performed in a manner consistent with data previously reported with the human keratinocyte (HaCaT) cell line. In all cases, the human skin models performed as well or better than the HaCaT cell model previously evaluated. In addition, this study identifies a clear unifying trigger that controls both the Nrf2/ARE pathway and essential biochemical events required for the development of ACD.

www.ncbi.nlm.nih.gov/pubmed/22494060

Environ. Health Perspect. 120:1150-1156 (2012) (DOI: 10.1289/ehp.1104709)

Profiling Environmental Chemicals for Activity in the Antioxidant Response Element Signaling Pathway Using a High Throughput Screening Approach

Sunita J. Shukla[1], Ruili Huang[1], Steven O. Simmons[2], Raymond R. Tice[3], Kristine L. Witt[3], Danielle VanLeer[1], Ram Ramabhadran[2], Christopher P. Austin[1], Menghang Xia[1]

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Oxidative stress has been implicated in the pathogenesis of a variety of diseases ranging from cancer to neurodegeneration, highlighting the need to identify chemicals that can induce this effect. The antioxidant response element (ARE) signaling pathway plays an important role in the amelioration of oxidative stress. Thus, assays that detect the up-regulation of this pathway could be useful for identifying chemicals that induce oxidative stress.

We used cell-based reporter methods and informatics tools to efficiently screen a large collection of environmental chemicals and identify compounds that induce oxidative stress. We utilized two cell-based ARE assay reporters, β-lactamase and

luciferase, to screen a U.S. National Toxicology Program 1,408-compound library (NTP 1408, which contains 1,340 unique compounds) for their ability to induce oxidative stress in HepG2 cells using quantitative high throughput screening (qHTS).

Our results support the robustness of using two different cell-based approaches for identifying compounds that induce ARE signaling. Together, these methods are useful for prioritizing chemicals for further in-depth mechanism-based toxicity testing.

www.ncbi.nlm.nih.gov/pubmed/22551509

- **Google Scholar**

Environmental Science and Pollution Research February 2013, Volume 20, Issue 2, pp 803-811

Mechanisms of toxicity of triphenyltin chloride (TPTC) determined by a live cell reporter array

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Triphenyltin chloride (TPTC), which has been extensively used in industry and agriculture, can occur at concentrations in the environment sufficient to be toxic. Here, potency of TPTC to modulate genes in a library containing 1,820 modified green fluorescent protein (GFP)-expressing promoter reporter vectors constructed from Escherichia coli K12 strains was determined. Responses were 1,230 and 97 times more sensitive than the acute median effect concentration (EC50) required to inhibit growth of cells, which demonstrated that this live cell array represents a sensitive method to assess toxic potency of chemicals. The 71 differentially expressed genes could be classified into seven functional groups. Of all the altered genes, three groups which encoded for catalytic enzymes, regulatory proteins, and structural proteins accounted for 28%, 18%, and 14% of all altered genes, respectively. The pattern of differential expression observed during this study was used to elucidate the mechanism of toxicity of TPTC. The genes rnC, cld, and glgS were selected as potential biomarkers for TPTC, since their expression was more than 2.0-fold greater after exposure to TPTC.

www.ncbi.nlm.nih.gov/pubmed/23128992

Chemical Research in Toxicology, Just Accepted Manuscript (DOI: 10.1021/tx4001754 Publication Date (Web): July 29, 2013)

A systematic study of mitochondrial toxicity of environmental chemicals using quantitative high throughput screening

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A goal of the Tox21 program is to transit toxicity testing from traditional *in vivo* models to *in vitro* assays that assess how chemicals affect cellular responses and toxicity pathways. Here, we evaluated the effect of chemical compounds on mitochondrial membrane potential in HepG2 cells by screening a library of 1,408 compounds provided by the National Toxicology Program (NTP) in a qHTS platform. Compounds were screened over 14 concentrations, and results showed that 91 and 88 compounds disrupted mitochondrial membrane potential after treatment for one or five h, respectively. Compounds were further assessed for mechanism of action (MOA) by measuring changes in oxygen consumption rate, which enabled identification of 20 compounds as uncouplers. This comprehensive approach allows for evaluation of thousands of environmental chemicals for mitochondrial toxicity and identification of possible MOAs. Copyright © 2013 American Chemical Society.

http://pubs.acs.org/DOI/abs/10.1021/tx4001754

Toxicological Sciences Volume 133, Issue 2 2013 Jun;:259-74. DOI: 10.1093/toxsci/kft075. Epub 2013 Mar 27.

Reactivity of Chemical Sensitizers Toward Amino Acids In Cellulo Plays a Role in the Activation of the Nrf2-ARE Pathway in Human Monocyte Dendritic Cells and the THP-1 Cell Line

Migdal C, Botton J, El Ali Z, Azoury ME, Guldemann J, Giménez-Arnau E, Lepoittevin JP, Kerdine-Römer S, Pallardy M.

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Allergic contact dermatitis resulting from skin sensitization is an inflammatory skin disease linked to the use of chemicals termed haptens. Chemical reactivity is necessary for a chemical to be a sensitizer, allowing both covalent binding to proteins and maturation of dendritic cells (DCs) by mimicking “danger signals.” The aim of this study was to evaluate how the reactivity of chemical sensitizers toward amino acids translates into a biological response using the activation of the nuclear factor-erythroid 2-related factor 2 (Nrf2) pathway, which was assessed by the induction of three Nrf2 target genes (ho-1, nqo1, and il-8) and Nrf2 protein accumulation.

Regression analysis revealed that ho-1 and nqo1 expressions were found to be associated with chemical sensitizer reactivity to cysteine, providing evidence of the importance of chemical reactivity, as a part of danger signals, in DC biology.

www.ncbi.nlm.nih.gov/pubmed/23535360

- **Terkko – Helsinki University Medical Library**

Regulatory Peptides Volume 180, 10 January 2013, Pages 33–42

Neuregulin-1β regulates tyrosine kinase receptor expression in cultured dorsal root ganglion neurons with excitotoxicity induced by glutamate

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Neuregulin-1 (NRG-1) signaling regulates neuronal development, migration, myelination, and synaptic maintenance. Three members of tyrosine kinase receptor (Trk) family, TrkA, TrkB, and TrkC, have been identified in DRG neurons. Whether NRG-1β and its signaling pathways influence the expression of these Trk receptors in DRG neurons is still unclear. In the present study, primary cultured DRG neurons were used to determine the effects of NRG-1β on TrkA, TrkB, and TrkC expression in DRG neurons with excitotoxicity induced by glutamate (Glu). The results indicated that in primary cultured DRG neurons with excitotoxicity induced by Glu, NRG-1β increased the expression of TrkA and TrkB their mRNAs, but not TrkC and its mRNA. Inhibitors (LY294002, PD98059) either alone or in combination blocked the effects of NRG-1β. NRG-1β may play an important role in regulating the expression of different Trk receptors in DRG neurons through the PI3K/Akt and ERK1/2 signaling pathways.

www.sciencedirect.com/science/article/pii/S0167011512002467

Frontiers in Physiology. 2012;3:462. DOI: 10.3389/fphys.2012.00462. Epub 2012 Dec 14.

Modeling drug- and chemical-induced hepatotoxicity with systems biology approaches.

Bhattacharya S, Shoda LK, Zhang Q, Woods CG, Howell BA, Siler SQ, Woodhead JL, Yang Y, McMullen P, Watkins PB, Andersen ME.

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We provide an overview of computational systems biology approaches as applied to the study of chemical- and drug-induced toxicity. We focus on toxicity of the liver (hepatotoxicity) - a complex phenotypic response with contributions from a number of different cell types and biological processes. We describe three case studies of complementary multi-scale computational modeling approaches to understand perturbation of toxicity pathways in the human liver as a result of exposure to environmental contaminants and specific drugs. One approach involves development of a spatial, multicellular "virtual tissue" model of the liver lobule that combines molecular circuits in individual hepatocytes with cell-cell interactions and blood-mediated transport of toxicants through hepatic sinusoids, to enable quantitative, mechanistic prediction of hepatic dose-response for activation of the aryl hydrocarbon receptor toxicity pathway. Simultaneously, methods are being developing to extract quantitative maps of intracellular signaling and transcriptional regulatory networks perturbed by environmental contaminants, using a combination of gene expression and genome-wide protein-DNA interaction data. A predictive physiological model (DILIsym™) to understand drug-induced liver injury (DILI), the most common adverse event leading to termination of clinical development programs and regulatory actions on drugs, is also described.

www.ncbi.nlm.nih.gov/pubmed/23248599

Inhalation Toxicology 2012 Dec;24(14):966-75. DOI: 10.3109/08958378.2012.742600.

Gene and protein responses of human lung tissue explants exposed to ambient particulate matter of different sizes.

Hoogendoorn B, Berube K, Gregory C, Jones T, Sexton K, Brennan P, Brewis IA, Murison A, Arthur R, Price H, Morgan H, Matthews IP.

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Exposure to ambient particulate air pollution is associated with increased cardiovascular and respiratory morbidity and mortality. The research was designed to investigate the effect of different size ranges of ambient particulate matter (PM) on gene and protein expression in an *in vitro* model. Eighteen different genes of the 84 on the PCR array were significantly dysregulated. Treatment with size 2 PM resulted in the greatest number of genes with altered expression, followed by size 1 and lastly size 3. ITRAQ identified 317 proteins, revealing 20 that were differentially expressed. Enrichment for gene ontology classification revealed potential changes to various pathways. This approach not only provides an investigative tool to identify possible causal pathways but also permits the relationship between particle size and responses to be explored.

www.ncbi.nlm.nih.gov/pubmed/23216157?dopt=Abstract#

Journal of Cellular Physiology Volume 228, Issue 6, pages 1359–1367, June 2013

Unraveling amyloid toxicity pathway in NIH3T3 cells by a combined proteomic and 1H-NMR metabonomic approach

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A range of debilitating human diseases is known to be associated with the formation of stable highly organized protein aggregates known as amyloid fibrils. The early prefibrillar aggregates behave as cytotoxic agents and their toxicity appears to result from an intrinsic ability to impair fundamental cellular processes by interacting with cellular membranes, causing oxidative stress and increase in free Ca2+ that lead to apoptotic or necrotic cell death. However, specific signaling pathways that underlie amyloid pathogenicity remain still unclear. The results of our work indicated that cell exposure to prefibrillar aggregates induces changes of the expression level of proteins and metabolites involved in stress response. The majority of the proteins and metabolites detected are reported to be related to oxidative stress, perturbation of calcium homeostasis, apoptotic and survival pathways, and membrane damage. In conclusion, the combined proteomic and 1H-NMR metabonomic approach, described in this study, contributes to unveil novel proteins and metabolites that could take part to the general framework of the toxicity induced by amyloid aggregates.

www.ncbi.nlm.nih.gov/pubmed/23192898

Cell Stem Cell Volume 11, Issue 5, 2 November 2012, Pages 620–632

Discovery of Inhibitors of Microglial Neurotoxicity Acting Through Multiple Mechanisms Using a Stem-Cell-Based Phenotypic Assay

Höing S, Rudhard Y, Reinhardt P, Glatza M, Stehling M, Wu G, Peiker C, Böcker A, Parga JA, Bunk E, Schwamborn JC, Slack M, Sterneckert J, Schöler HR.

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Stem cells, through their ability to both self-renew and differentiate, can produce a virtually limitless supply of specialized cells that behave comparably to primary cells. We took advantage of this property to develop an assay for small-molecule-based neuroprotection using stem-cell-derived motor neurons and astrocytes, together with activated microglia as a stress paradigm. Here, we report on the discovery of hit compounds from a screen of more than 10,000 small molecules. These compounds act through diverse pathways, including the inhibition of nitric oxide production by microglia, activation of the Nrf2 pathway in microglia and astrocytes, and direct protection of neurons from nitric-oxide-induced degeneration. We confirm the activity of these compounds using human neurons.

www.ncbi.nlm.nih.gov/pubmed/23064101

Biochimica et Biophysica Acta 2013 Oct;1833(10):2311-21. DOI: 10.1016/j.bbamcr.2013.05.021. Epub 2013 Jun 1.

Protein kinase CK2-dependent phosphorylation of the human Regulators of Calcineurin reveals a novel mechanism regulating the calcineurin-NFATc signaling pathway.

Martínez-Høyer S, Aranguren-Ibáñez A, García-García J, Serrano-Candelas E, Vilardell J, Nunes V, Aguado F, Oliva B, Itarte E, Pérez-Riba M.

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Cyclosporine A and FK506 produce immunosuppression by blocking calcineurin phosphatase activity and consequently activation of cytosolic Nuclear Factor of Activated T-cell (NFATc) transcription factor. Due to the chronic toxicity associated with their administration, the development of more specific immunosuppressants is currently an important unmet medical need. Molecular modeling studies have led us to identify a positively charged interaction site on the surface of calcineurin where the phosphorylated serine residue of the CIC motif would normally locate. Finally, we have also identified RCAN3 as a new phosphoprotein with multiple phosphorylation sites. Therefore, our findings reveal for the first time a novel molecular mechanism underlying the regulation of calcineurin-NFATc signaling by means of phosphorylation of the CIC motif of RCAN proteins. Copyright © 2013. Published by Elsevier B.V.

www.ncbi.nlm.nih.gov/pubmed/23732701

Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids Volume 1831, Issue 8, August 2013, Pages 1344–1356

A signaling cascade mediated by ceramide, src and PDGFRβ coordinates the activation of the redox-sensitive neutral sphingomyelinase-2 and sphingosine kinase-1

Cinq-Frais C, Coatrieux C, Grazide MH, Hannun YA, Nègre-Salvayre A, Salvayre R, Augé N.

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Stress-inducing agents, including oxidative stress, generate the sphingolipid mediators ceramide (Cer) and sphingosine-1-phosphate (S1P) that are involved in stress-induced cellular responses. The two redox-sensitive neutral sphingomyelinase-2 (nSMase2) and sphingosine kinase-1 (SK1) participate in transducing stress signaling to ceramide and S1P, respectively; however, whether these key enzymes are coordinately regulated is not known. We investigated whether a signaling link coordinates nSMase2 and SK1 activation by H_2O_2. In

mesenchymal cells, H_2O_2 elicits a dose-dependent biphasic effect, mitogenic at low concentration (5 μM), and anti-proliferative and toxic at high concentration (100 μM). The results also showed that the toxicity of high H_2O_2 concentration was comparable in control and nSMase2-deficient cells. Taken together the results identify a tightly coordinated nSMase2/SK1 pathway that mediates the mitogenic effects of H_2O_2 and may sense the degree of oxidative stress.

www.ncbi.nlm.nih.gov/pubmed/23651497

International Journal of Molecular Sciences 2013 Mar 11;14(3):5633-49. DOI: 10.3390/ijms14035633.

Global Gene Expression Profiling Reveals Functional Importance of Sirt2 in Endothelial Cells under Oxidative Stress.

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The NAD+-dependent deacetylases Sirt1 and Sirt2 mediate cellular stress responses and are highly expressed in vascular endothelial cells. In contrast to the well-documented protective actions of Sirt1, the role of endothelial Sirt2 remains unknown. Using cDNA microarray and PCR validation, we examined global gene expression changes in response to Sirt2 knock down in primary human umbilical vein endothelial cells under oxidative stress. We found that Sirt2 knock down changed expression of 340 genes, which are mainly involved in cellular processes including actin binding, cellular amino acid metabolic process, transmembrane receptor protein serine/threonine kinase signaling, ferrous iron transport, protein transport and localization, cell morphogenesis, and functions associated with endosome membrane and the trans-Golgi network. Our results may provide a basis for future studies aiming to dissect the specific signaling pathway(s) that mediates specific Sirt2 functions in endothelial cells.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3634502/