P25: Omics

P25-01
Bioinformatics-based cancer research have wide toxicological applicability

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We constructed an omics data-driven pipeline for cancer biomarker discovery considering that it should be applicable also to toxicogenomics studies. As proof of concept, transcriptomics (microarray) and bioinformatics tools were applied to assess differentiation-induction, serving as a common toxicological outcome in toxicant-exposed epithelia. Competent and non-competent human keratinocytes were studied, including normal and immortalized oral keratinocytes (NOK and SVpgC2a, respectively). The immortal cells exhibited consistent absence of differentiated morphological features, an effect that was coupled to many-fold elevated cloning ability and saturation density. Transcriptomics analysis implicated 48 gene ontology (GO) categories, eight molecular networks (MNW) and 10 key regulator genes (KRG) in density-dependent differentiation of NOK, all of which remained non-regulated in SVpgC2a. The SVpgC2a versus NOK transcriptome enriched altogether 52 GO categories, 18 MNW and 39 KRG, several of which associated to epithelial–mesenchymal transition. Assessment of the above gene sets, including all differentially expressed genes, relative (and sequentially) to two training data sets of cancer tissue samples identified a 4-gene signature (COX7A1, MFAP5, MPDU1 and POLD1) that predicted poor patient survival. Independent validation in another gene set confirmed the predictive ability of the 4-gene signature for poor outcome. The present study defined for the first time the spectrum of genes that can couple broadly to differentiation induction, a primary biological fate in toxicity studies. The overall approach taken for bioinformatics-based biomarker discovery relative to cancer prognosis is potentially widely applicable also to computational toxicology.

doi:10.1016/j.toxlet.2012.03.580

P25-02
The identification of hypothyroid effects of amiodarone by metabolomics

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BASF and metanomics have been developing the metabolite profiles (metabolome) in rat serum in the context of a 28 day study, with blood sampling at days 7, 14 and 28. With the metabolome and toxicity profile of over 500 chemicals we have established a comprehensive database (MetaMapÒTox). The metabolic profile consists of ca. 300 analytes, sets of which were combined to identify common metabolite level changes (metabolite patterns) associated with defined toxicological modes of action (MOAs). Among these metabolite patterns, we have established some to differentiate between a direct effect (thyroperoxidase inhibition) and an indirect effect (increased thyroid hormone conjugation by microsomal liver enzyme induction) on thyroid hormone homeostasis. Amiodarone, an antiarrythmetic drug, has been reported to cause thyroid changes in humans (Cohen-Lehman, J. et al. Nat Rev Endocrinol 6, 34–41, 2010). Already in 2007, we had included amiodarone in our project with the intention to establish a metabolic pattern for its pharmaceutical effects. We now retrospectively analyzed its metabolome for indications of thyroid effects. We clearly identified an indirect mechanism, but also found indications for a further mode of action on the thyroid, that was associated with a direct effect, surprisingly however, without reducing thyroid hormone levels. A possible explanation is that amiodarone, due to its high iodine content, interferes with thyroid homeostasis mechanisms. Other potential causes for the thyroid effects include alterations in deiodinases activity, an interaction with the thyroid receptor or a displacement of thyroid hormone from carrier proteins in the blood.

doi:10.1016/j.toxlet.2012.03.581

P25-03
Identification of liver toxicity by combining in vitro cell systems and metabolomics

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The use of alternatives to animal testing has increased in the last years, not only due to new regulations, such as REACH or the 7th Amendment of the cosmetic directive, but also because they can be used for toxicological screening during compound development. The use of state-of-the-art technologies in combination with cellular in vitro models represents a new opportunity to obtain complete information about the toxicological mode of action of compounds in commerce and most importantly compounds under development. In vitro cell culture coped to omics, technologies represent promising alternatives, since on one hand, it is possible to obtain quantitative information about the multi-parametric metabolic response of the cellular systems in normal and patho-physiological conditions in a high throughput fashion and on the other hand, it does not require animal testing. Therefore, the combination of both systems represents an effective tool to analyze toxicological effects of chemical compounds. We have exposed HepG2 cells to different hepatotoxicants and have subsequently analyses the metabolome of the cells. The obtained metabolic fingerprint allowed for a clear distinction between different toxicological modes of action. Thus, our preliminary results seem promising with respect to a future identification of metabolic fingerprints induced by compounds in in vitro models.

doi:10.1016/j.toxlet.2012.03.582

P25-04
Association of the divalent metal transporter 1 gene polymorphism with thalassemia

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Thalassemia is the most common hereditary blood disorders worldwide especially Asia, Mediterranean, Middle East and Africa. Thalassemia includes of two main types: alpha and beta which have

different genetic defects, disease manifestation, and, whereupon different prenatal diagnosis and management. Patients with thalassemia can get an overload of iron in their bodies, either from the disease itself or from frequent blood transfusions. Overload iron is responsible for a series of complications include involvement of the heart, liver and endocrine glands. Divalent metal transporter 1 (DMT1) play important roles in intestinal iron absorption, erythroid iron utilization, accumulation and transfer. The aim of the present study was to investigate relationship between the DMT1 gene polymorphism and thalassemia.

In this study, we collected 50 blood samples from genetically unrelated thalassemia patients and 50 blood samples from healthy unrelated controls. In order to screen for DMT1 gene polymorphism standard PCR-RFLP technique was used. While the distributions of DMT1 CC, CA and AA genotypes in thalassemia patients were 30.0%, 46.0% and 24.0%, respectively, these ratios were found in control group as 56.0% for CC, 32.0% for CA and 12.0% for AA genotypes. As a result, statistically significant differences were determined between the patient and control groups in DMT1 genotype distribution ($p < 0.05$). It is known that in thalassemia patients with the DMT1 atypical genotypes, intestinal Fe absorption decreases thus Fe does not overload in their bodies. These thalassemia patients may be more advantageous against to severity of iron overload than with typical and heterozygote genotypes of DMT1.

doi:10.1016/j.toxlet.2012.03.583

P25-05
Transcriptomic and functional alterations during renal epithelial maturation

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Purpose: The proximal tubule is very sensitive to xenobiotics, which can have serious consequences on homeostasis. Injury to epithelial cells alters cell to cell contacts and promotes proliferation and tissue repair. In order to better understand these processes, we characterized temporal alterations of human renal proximal tubule cultures during monolayer formation. *Methods*: Primary human proximal tubule cells and the human RPTEC/TERT1 cell line were cultured in hormonally defined DMEM/F12 for 16 days, allowing for monolayer formation. At day 1 and then every third day, cells were harvested for RNA isolation and cell cycle analysis. In addition, supernatants were collected to analyse glycolysis rates. *Results*: Using Illumina HT-12 whole-genome gene expression arrays, 1238 differently expressed genes common to both cell types were identified. The time of culture had a large impact on the gene expression, stabilising around day 13 after seeding concomitantly with retardation in G0/G1 cell cycle phase. Enhanced cell border localization of adherents and tight junction proteins was observed, as well as temporal increases at both transcript and protein levels. Expression of genes involves in energy metabolism (glycolysis, TCA, oxidative phosphorylation and fatty acid metabolism) were altered, whereas glycolytic rates decreased over time. Interestingly, oxygen consumption was higher in proliferating cells than in matured cells, but matured cells exhibited a higher oxidative capacity. *Conclusion*: The generated data set of temporally differently expressed genes will be useful to identify mechanistically linked biomarkers of epithelial cell injury processes.

doi:10.1016/j.toxlet.2012.03.584

P25-06
Sharing dose response data and analysis across the SEURAT-1 cluster

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The ToxBank consortium is developing a series of infrastructure projects to support the activities across SEURAT-1 including a data warehouse. This warehouse is a web accessible shared repository of know-how and experimental results to support the development of a replacement for in vivo repeated dose toxicity testing. The information within the ToxBank data warehouse will be uploaded from the research activities of the cluster partners as well as relevant data and protocols from other sources, such as public databases, to enable a cross-cluster integrated data analysis leading to the prediction of repeated dose toxicity.

During the first year of the project, the ToxBank consortium implemented a detailed requirements gathering exercise. A series of interviews covered a variety of activities including cell differentiation, cell engineering, biomarker identification, dose response analysis, toxicity testing, omics experiments, chemical analysis, and cell banking. The ToxBank data warehouse was then designed to support the activities across the entire SEURAT-1 cluster and technology selected on which the data warehouse will be built.

As a first use case, the ToxBank consortium in collaboration with DKFZ who are partners in the DETECTIVE consortium, have developed a storyboard to illustrate how dose response protocols and data generated could be recorded, uploaded and used to support the activities across the entire cluster. The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement no. [267042]. The research leading to these results has received financing from Colipa.

doi:10.1016/j.toxlet.2012.03.585

P25-07
Oxidative modification leads to the left ventricular dysfunction in cardiomyopathy

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Purpose: Given the hypothesis that an inflammation enhanced by reactive oxygen species (ROS) accumulation plays an essential role in the progression of cardiovascular diseases, we aimed to identify and characterize ROS-induced carbonyl modification of myocardial proteins involved in the cardiac dysfunction, using proteomics analysis. *Methods*: Using the two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) and 2D-oxyblot analysis combined with MALDI-TOF/TOF tandem mass spectrometry, we compared the changes in expression and modification of proteins in the whole left ventricle (LV) between control hamsters, dilated cardiomyopathy hamsters (TO-2), and hypertrophy