Session I-12: Epigenetics and its increasing relevance in toxicology and risk assessment

Session I-12: Oral presentations

I-12-718

An introduction to epigenetics

M. Vinken
Vrije Universiteit Brussel, Brussels, Belgium
mvinken@vub.ac.be

The term “epigenetics” was coined in the 1940s and denotes the heritable changes in the phenotype or gene expression patterns caused by mechanisms other than changes in the underlying DNA sequence. Since its introduction, the epigenetics research field has experienced a true boost, particularly in the last two decades, with the in-depth characterization of the determinants of the epigenome and the establishment of cutting-edge technology to study these features as milestones. The major drivers of the epigenetic machinery, including DNA methylation, histone modifications and regulation by non-coding RNA species, will be briefly discussed in the current presentation. Furthermore, the increasing relevance of epigenetics for both the study and practice of toxicology will be illustrated.

Session I-12: Poster presentations

I-12-129

Computational modelling and the reduction of animal testing in toxicology studies

G. Edmund and B. Howlin
University of Surrey, Guildford, UK
g.edmund@surrey.ac.uk

Cytochrome P450 enzymes (CYP450) are central to drug metabolism, carrying out the metabolism of 75% of known drugs in current clinical use. Important effects such as adverse drug reactions and genetically determined differences in drug toxicity and efficacy depend on CYP450 activity, yet this cannot be easily predicted from protein structures alone and is often found through the use of animal models.

Computational methods allow for a better understanding of the molecular determinants of reactivity and specificity, potentially contributing significantly to both drug development, assisting in the prevention of the growing and expensive problem of late stage drug failures (which often occur due to CYP450 mediated ADME-Tox properties), and in the reduction (and eventual replacement) of animal testing at this stage of drug development.

Our aim is to provide validated computer models of the CYP450 enzymes that can be used in *in silico* screening of new drug molecules, thereby greatly reducing the need for animal testing in toxicity studies. By using 3D homology modelling and
Hepatocyte-based models have become standard in vitro tools to evaluate pharmaco-toxicological characteristics of compounds. However, primary hepatocytes in culture cope with the progressive deterioration of their specific in vivo phenotype, including xenobiotic biotransformation capacity, which largely restricts their application to short-term in vitro studies. To stabilize hepatocyte cultures, scientists have tried to mimic the natural hepatoctye micro-environment in culture through the use of extracellular matrix components, adding soluble medium components or co-culturing with other cell types. However, this has only led to a slight improvement of the viability and the preservation of the differentiated phenotype. Therefore, new strategies need to be explored. Since epigenetic mechanisms such as histone acetylation and/or DNA methylation play a predominant role in the regulation of hepatic gene expression, interfering with these pre-transcriptional processes could aid in developing a long-term hepatocyte model for in vitro testing and screening purposes. Indeed, we were the first to show that inhibitors of these processes, including Trichostatine A (TSA) and 5'-aza-2'-deoxycytidine, respectively, (synergistically) cause proliferative blocks, counteract spontaneous apoptotic cell death, and promote functional and morphological differentiation of primary hepatocytes in culture. Moreover, it was recently also found that TSA up- and down regulates microRNA (miR)-379 and miR-122, and miR-143, respectively, which all could probably be related to the inhibitory effects of TSA on hepatocellular proliferation. In conclusion, our data indicate that classical epigenetic regulators either alone or in combination with modulators of miRNA species, represent innovative tools to develop more stable and functional primary hepatocyte cultures.

A test strategy to detect developmental toxicants that affect neural development using human embryonic stem cells

Doerenkamp-Zbinden Chair for in vitro toxicology and biomedicine, University of Konstanz, Konstanz, Germany
marcel.leist@uni-konstanz.de

Developmental neurotoxicity (DNT) is caused by exposure to toxicants during sensitive periods of neurodevelopment and can lead to cognitive, sensory or behavioural deficits, persisting long after removal of the original stimulus. How the “memory” of the early developmental exposure is stored is unclear yet, and information on developmental neurotoxic effects of most chemicals is still very sparse. To address these issues, we established a test system based on human pluripotent cells with the potential to differentiate towards neural cells. We profiled not only the changes of mRNAs, but also of miRNAs and obtained a profile of a large set of chromatin modifiers. The large changes in the expression of chromatin modifiers were corroborated by altered nuclear staining patterns of histone modifications. To test the sensitivity of the earliest phase of neurodevelopment to epigenetic modifying chemicals, we used human embryonic stem cells, which were differentiated to Pax6-positive neural precursor cells. In this system we investigated the role of various signalling pathways for neural differentiation and how toxicants might interfere with those pathways. The cells were also exposed to epigenetic modulators. As read-out, we used mRNA expression levels of markers specific for certain neurodevelopmental stages and flow cytometry of a reporter gene, and identified pronounced developmental toxicity in the absence of cytotoxicity.