



Session V-5: Developments in stem cell research as the basis for sustainable availability of differentiated human cells and tissues

Session V-5: Oral presentations

V-5-422

Focus on stem cells as sources of human target cells for *in vitro* research and testing

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Our knowledge with respect to stem cell technology has been growing steadily. This is not surprising as human stem cells could play a major role in regenerative medicine, offering hope for human cells for disease research and therapy. Sources of stem cells consist not only of embryonic pluripotent cells, but also of multipotent adult stem cells derived from a variety of organs. For clinical purposes, most interest goes to induced pluripotent stem cells, which can be converted into desired cell types by directed differentiation and reprogramming. This huge clinical interest is also a boost for other fields including drug development, *in vitro* modeling and pharmacotoxicology. Indeed, human stem cell-derived target cells stimulate the introduction of functional *in vitro* models relevant for the human situation.

They could be applied in research and in regulatory testing. Of particular interest is the generation of human hepatocytes. They are responsible for major liver functions, including phase I and phase II drug metabolism. The latter often is at the origin of drug activation, making the liver a toxicity target. Unlimited access to human hepatocytes could generate *in vitro* models to study liver function, drug metabolism and the different mechanisms underlying drug induced liver injury, being a major cause of drug attrition. They are important in drug development in general, for screening purposes and in particular in the risk assessment of new biological entities for which human-derived models are crucial.

V-5-702

Dermal stem cells: An accessible multipotent precursor with potential application for drug screening and therapeutics

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Stem cell-based therapies hold great promise for repair and functional restoration following tissue injury and disease. Skin-derived precursors (or "SKPs") are a novel, multipotent somatic stem cell that resides within the mammalian dermis. SKPs persist within the skin throughout adulthood and yet intriguingly, exhibit many similarities to embryonic neural crest stem cells (NCSCs). For example, SKPs give rise to both neural and meso-

dermal cell types, exhibit similar gene expression profiles and the former appear biased to peripheral nervous system fates. Here I will summarize our current understanding of the biological origin of SKPs and specifically the potential therapeutic utility of SKPs as a highly accessible, autologous and renewable source of neural crest-like precursors.



V-5-429

A stem cell based test battery to detect developmental neurotoxicants

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In order to identify compound effects on different steps and mechanisms involved in neurodevelopment, we designed a test battery consisting of different model systems. In addition we performed a literature search to assemble a list of relevant test compounds of known toxicity, developmental neurotoxicity or proven absence of toxicity. The following assay systems were used: (1) Murine embryonic stem cells (mESC) were converted into terminally differentiated neurons. (2) Conditionally-immortalized human neural precursor cells (LUHMES) were differentiated to a homogeneous population with a complex neurite network and typical biochemical and morphological features of dopaminergic neurons. (3) Human embryonic stem cells differentiated to central nervous system neural precursors or to peripheral nervous system neural crest stem cells.

We used different readouts for compound effects, all measured in a concentration range not causing direct cytotoxicity.

Transcript levels were used to characterize disturbed differentiation patterns on the basis of up to 80 endpoints. In addition, functional endpoints were chosen to reflect neurite outgrowth capacity, cell migration and the turnover of neurotransmitters. For instance methylmercury affected neuronal maturation and neurotransmitter uptake at much lower concentrations than required for neurite toxicity. It also affected the migration of stem cells at concentrations lower than those required to inhibit proliferation. This approach allowed profiling of different compounds. A new class investigated was that of epigenetic modifiers. The human cell systems were extensively characterized for chromatin modifying factors, and for instance histone deacetylase inhibitors affected the differentiation pattern towards neuronal precursors.

V-5-410

Reconsidering pluripotency tests: Do we still need teratomas?

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The production of teratomas in immunodeficient mice is regarded as the “gold standard” assay for pluripotency. This assay has been used to demonstrate the pluripotency of embryonic stem cells, induced pluripotent stem cells (iPSC) and other pluripotent cells. However, the teratoma assay raises two main issues concerning animal welfare: (i) the inoculation of animals with potentially malignant tumors, and (ii) the breeding of genetically deficient experimental animals. Both of these issues are associated with suffering of the animals. To explore pluripotency testing in the context of the 3Rs, the German Foundation for the Promotion of Alternatives to Animal Testing (*Stiftung zur Förderung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen, set*) organized an expert work-

shop in December 2010. This presentation will demonstrate the results of this workshop. Several alternatives to the mouse teratoma assay were discussed, including the directed differentiation of ES and iPSC cells into organotypic cells, expression of pluripotency-associated markers such as TRA-1-60, DNMT3B, REX1 that correlate well with the teratoma forming potential of ES and iPSC cells, epigenomic footprints, such as DNA methylation, and histone modifications. Each of these assays is capable of addressing one or several aspects of pluripotency. It is imperative that more research be performed in order to standardize such alternative tests. Simple, robust, reliable, standardised tests need to be developed to facilitate the testing of pluripotency of new and existing cell lines.



V-5-184

Characterization of developmental changes and electrophysiological functions in contracting cardiomyocytes derived from human iPS cells

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Cardiomyocytes derived from human induced pluripotent stem cells (hiPS-CMs) promise to become a useful *in vitro* tool for assessing cardiotoxicity, including QT prolongation, and to contribute to a reduction in animal use in drug discovery. Thus, clarifying their functional properties and the developmental changes in their transcriptional expression pattern is very important for confirming their suitability for safety assessment of drug candidates. In this study, we characterized the properties of contracting hiPS-CMs and assessed their functionality using gene expression analysis and whole-cell patch-clamp recordings. We used the human induced pluripotent stem cell line 201B7 (Takahashi et al., 2007) and the embryoid body method for differentiation. Beating colonies appeared around day 8 of differentiation. In the contracting areas at the later stage (day 38), mRNA expressions of cardiac-related ion channels and markers were detected. After contraction started, expression

levels of some ion channel mRNAs were upregulated gradually according to the time in culture. These changes were in agreement with those of cardiomyocytes from human embryonic stem cells and suggest that beating hiPSC-CMs mature progressively. Pharmacological responses of major cardiac ion currents and action potential duration to each known ion channel blocker in hiPS-CMs at the later stage were similar to those in human cardiomyocytes. These results reveal the functional suitability of hiPS-CMs for QT risk assessment and indicate that the developmental profiles of their ion channel mRNA expressions reflect appropriate differentiation and maturation of hiPS-CMs for safety assessment of drug candidates.

Reference

Takahashi, K., Tanabe, K., Ohnuki, M. et al. (2007). *Cell* 131, 861-872.

V-5-703

Skin-derived precursor cells – a promising source for hepatic progeny

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Withdrawal of promising drug candidates is often triggered by the detection of hepatotoxicity in (pre)clinical studies. The availability of reliable *in vitro* screening models capable of detecting hepatotoxicity and in particular chronic liver toxicity in an early stage of the drug development process is thus of utmost importance for the pharmaceutical industry. Today, however, most existing liver-based models suffer from phenotypic instability and are rodent-derived, making them not fully representative of the human situation. The development of a model based on easily obtainable human adult stem cells could overcome this problem. A promising stem cell source is the human skin-derived precursor cell (hSKP), a multipotent neural-crest related precursor capable of generating neuronal, glial and mesodermal progeny.

In the present study, we show that these cells are able to undergo endodermal differentiation. More specifically, upon se-

quential and gradual exposure to hepatogenic factors, hSKP differentiate into immature hepatocyte-like cells expressing foetal and mature hepatic markers in a time-dependent manner, reflecting the hepatogenesis *in vivo*. Upon intrasplenic transplantation into the uPA^{+/+}-SCID mouse model, hSKP-derived hepatic cells are able to migrate to, engraft and survive in the diseased mouse liver for more than 10 weeks. Furthermore, hSKP-derived hepatic cells are able to immunomodulate the immune response both in the presence and absence of inflammatory conditions, indicating their high potential as a promising source for *in vivo* transplantation as both a hepatocyte-like or supportive cell type. To conclude we can say that hSKP are a promising multipotent stem cell source to generate hepatic progeny for both *in vitro* and *in vivo* applications.



Session V-5: Poster presentations

V-5-181

Comparison of three kinds of ES cells using two and three-dimensional culture systems

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In 1997, Spielmann et al. developed the embryonic stem cell test (EST), which is an *in vitro* embryotoxicity test protocol that can be used to estimate the risk of embryotoxicity of chemical substances quickly compared to the conventional methods that involve animal experiments. The EST has been evaluated in a validation study funded by ECVAM, in which two other *in vitro* embryotoxicity tests (micromass test, whole embryo culture test) were validated against a set of test chemicals characterized by high levels of *in vivo* embryotoxicity in laboratory animals and humans.

In the EST protocol, mouse ES-D3 cells have been listed as the only ones available. If other kinds of ES cells become available, the experimental application will be of greater usefulness. We conducted a comparison between non-feeder ES-D3 cells

and EL M3 cells or ES-R1-EGFP B2/EGFP cells requiring feeder cells, to explore the experimental possibility of using ES cells requiring feeder cells.

As the present results with ES-D3 and EL M3 cells were similar to those obtained under the two-dimensional condition, these two kinds of cell are thought to be equally available under the present three-dimensional conditions. On the other hand, because ES-R1-EGFP B2/EGFP cells did not show any pulsation at all in the three-dimensional culture, other experimental conditions for the three-dimensional culture method need to be established with those cells. In addition, it was suggested that similar results could be obtained with EL M3 cells requiring feeder cells for cultivation compared to those with ES-D3 cells.

V-5-318

Egg yolk extract – a novel alternative to Fetal Bovine Serum in goat stem cell culture media

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Fetal bovine serum (FBS) is a regular component of animal cell culture media. FBS is harvested from the fetuses of pregnant cows during slaughter. Being alive at the time of blood collection, these fetuses are exposed to severe pain and stress. The amount of FBS produced for the world market is approximately 500,000 litres per year. For this, more than 1,000,000 bovine fetuses are harvested and this number is steadily increasing. Scientific and ethical concerns exist regarding the use of FBS in *in vitro* cell culture. Stem cell research is a fast developing area in life science. Across the world several studies are in progress to find a suitable medium which can maintain the immortality of stem cells. Therefore, the present study was conducted to exploit

the potential of Egg Yolk Extract (EYE) as a possible alternative to FBS in goat fetal stem cell culture media. EYE was prepared from fresh unfertilized chicken eggs after proper processing. Goat fetal stem cells were isolated and cultured *in vitro* adopting standard procedure. EYE was used at a concentration of 4% in a standard stem cell culture medium vs. control medium with 10% FBS. In the EYE supplemented media, fetal stem cells had normal growth and multiplication and produced maximum number of clones in subsequent cultures. These clones were later characterized with stem cell specific markers, i.e. Oct4, Nanog and Sox2. The study proved that EYE can be used as an efficient alternative to FBS for culturing goat stem cells.

V-5-477

A human neuronal cell line for the substitution of transgenic neurodegeneration models

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Research in the field of neurodegeneration such as Parkinson's Disease (PD) or Alzheimer's Disease (AD) requires the selective manipulation of genes of interest. Transgenic mouse models of PD-related genes (alpha-synuclein, tyrosine hydroxylase, DJ-1), or AD-related genes (APP, BACE1, gamma-secretase), were successfully generated in recent years. Apart from the labor- and cost-intensive procedures, both in the generation and application of these *in vivo* models, the transgenic mouse approach has led to a significant increase in the number of animal experiments.

We herein introduce the conditionally immortalized human neuronal cell line LUHMES as a new *in vitro* alternative to currently established primary cell, cell line, or *in vivo* models. We have established different protocols that allow differentiation into a dopaminergic phenotype for PD studies, as well as for

a dopamine-independent neuronal phenotype for AD research. LUHMES in combination with PD-toxins (MPP+ or methamphetamine), resembles key features observed in the PD brain, including oxidative, proteasomal, and metabolomic stress. Alternatively, the cells can directly be applied as model for AD, as they endogenously express amyloid precursor protein (APP) and the proteolytic machinery for the formation of amyloid beta (A-beta) peptide.

We have furthermore established a lentiviral system for the overexpression of alpha-synuclein, tyrosine hydroxylase, as well as APP and BACE1 in LUHMES, knock-down of all these targets is achieved by siRNA. The LUHMES model hence represents a robust and highly reproducible *in vitro* model on the basis of human neuronal cells that can also be applied in high throughput screening programs.

V-5-514

Generation of post-mitotic neurons from the human LUHMES cell line

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We characterized phenotype and function of a fetal human mesencephalic cell line (LUHMES) as a neuronal model system. Neurodevelopmental profiling of the proliferating stage (d0) of these conditionally-immortalized cells revealed neuronal features, expressed simultaneously with some early neuroblast and stem cell markers. An optimized 2-step differentiation procedure, triggered by shut-down of the myc transgene, resulted in uniformly post-mitotic neurons within 5 days (d5). This was associated with downregulation of some precursor markers and further upregulation of neuronal genes. Neurite network formation involved the outgrowth of 1-2, often >500 μm long projections. They showed dynamic growth cone behavior, as evidenced by time-lapse imaging of stably GFP-overexpressing cells, and grew neurites. This extension was specifically inhibited by a

set of tool compounds in a concentration-dependent manner. Voltage-dependent sodium channels and spontaneous electrical activity of LUHMES continuously increased from d0 to d11, while levels of synaptic markers reached their maximum on d5. The developmental expression patterns of most genes and of the dopamine uptake- and release-machinery appeared to be intrinsically predetermined, as the differentiation proceeded similarly when external factors such as dibutyryl-cAMP (cAMP) and GDNF were omitted. Only tyrosine hydroxylase required the continuous presence of cAMP. In conclusion, LUHMES are a robust neuronal model with adaptable phenotype and high value for neurodevelopmental studies, disease modeling and neuropharmacology.