Session 1: Alternative experimental models to improve genetic toxicity testing

Chairs

Dr. Marilyn Aardema (BioReliance)

Dr. Stefan Pfuhler (Procter & Gamble)

ILSI Health and Environmental Sciences Institute

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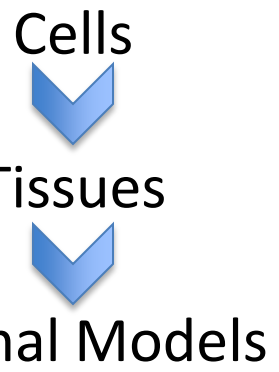
Agenda

- **Introduction** – *Dr. Marilyn Aardema (BioReliance, USA)*
- **Overview of the use of 3-dimensional tissue constructs for genotoxicity testing**
  - *Dr. Stefan Pfuhler (Procter & Gamble, USA)*
- **Development of in vitro toxicity testing using hepatocytes differentiated from human stem cells**
  - *Dr. Seiichi Ishida (NIHS, Japan)*
- **Humanized models in toxicology and their applications to hazard characterization and risk assessment**
  - *Dr. Darrell Boverhof (Dow Chemical, USA)*
- **Session 1 Discussion**—SWOT analyses

Rationale for the Topics in Session 1

- **New technologies are available/being developed**
  - Need to consider how to apply these in the field of genetic toxicology
  - What is needed to foster their use globally
  - What can ILSI-HESI do to facilitate use?
- **One major focus is on more biologically relevant human-based models**
  - Stem cells
  - 3D
  - Humanized animal models



Tool or assay

SWOT analysis: Name of the assay

Strengths

- What advantages over other technologies does the assay you will describe offer?
- The assay you will describe being used in laboratories other than yours/ the technology available to other potentially interested investigators?
-Large data base (specify classes of compounds)?
- Others

Weaknesses

- The assay you will describe not being used in laboratories other than yours/ the technology not available to other potentially interested investigators?
-Limited data base (specify classes of compounds)?
- Others

Opportunities

- How do you envision this method be used in regulatory research scenarios?
- At what stage of hazard/risk assessment?
- Collaborative effort needed/recommended?
- What questions does data from this assay answer about the qualitative or quantitative hazard (genotoxic risk?) for the individual compound being tested?
-Others

Threats

- What barriers do you see to wider acceptance of this method?
- Does patent protection limit other's access to the technology?
- Others

Strengths

- What advantages over other technologies does the assay you describe offer?
- How widely is the assay used?
- How large is the data base (specify classes of compounds)

Weaknesses

-What is missing from the assay/approach?

Opportunities

- How do you envision this method being used in regulatory research scenarios?
  - Hazard assessment?
  - Rish assessment?
- Is a collaborative effort needed/recommended to gain wider use?
- What questions does data from this assay answer about the qualitative or quantitative hazard (genotoxic risk?) for the individual compound being tested?

Threats

- What barriers do you see to wider acceptance of this method?
- Does patent protection limit other's access to the technology?
- Others

What can ILSI-HESI Do?

- Hold more meetings/workshops
- IVGT could interface with Risk21
- Should we evaluate same compounds to bridge in vitro and in vivo using these assays
- Should ILSI make recommendations on whether a technology can replace previous assays
- Cell bank – include cells to be used for developing models

Discussion

- Physiological anchoring
  - Key to interpreting in vitro assays – are assay conditions reflective of in vivo physiological parameters (also prolif., metabolic parameters); need to understand relevance
- What is the endgame – are we better off than previously
- What question or we trying to answer? (e.g., are we predicting cancer, understanding animal/human differences)
- What are gaps in current way of doing RA?
- For RA – only use guideline studies
  - Test battery locks us in
  - Single battery does not answer all questions – need to understand questions – test battery is nice way to start when you don't know anything about the compound
  - After doing test battery – move on to other studies to better understand compound

Tool or assay

SWOT analysis: 3D tissue constructs

Strengths

- **More closer to 'in vivo' conditions**
  - **Closer to in vivo means prolif. – more difficult for genetox (trading off false positives for false negatives) – could be an opportunity to develop better models**
  - **Model-specific approach; tissue should prolif. as normal as possible – but respond appropriately to toxic insult**
- **Cells are of human origin and function more organ-like in terms of cell viability, proliferation, differentiation, morphology, gene and protein expression**
- **Large interest in assay, used by increasing no. of laboratories in various geographies**
- **(Pre) Validation exercise ongoing for 3D skin**
- **3D skin / liver – testing for known genetox endpoints – surrogate model -**

Tool or assay

SWOT analysis: 3D tissue constructs

Weaknesses

- **More difficult to handle and more expensive**
- **High throughput possible only for low-complexity models**
- **Various levels of supporting data available - minimal to moderate**
- **Used in limited no of labs**
- **Assays at stage of early development for some models**

Tool or assay

SWOT analysis: 3D tissue constructs

Opportunities

- 3D constructs have the potential to serve as 2nd tear assays to follow up on positives from 1st tier
- Collaborative efforts recommended for relevant tissues for which no such efforts are currently ongoing (liver, lung)
- May be useful also to help bridge gap between in vitro and in vivo in terms of quantitative risk assessment
    - Don't generally do quant RA on in vitro tests
    - Could define internal dose – but need to normalize data
- tools to dive in deeper – (not replacement assays)
    - Or use for specific chem class where we know that existing assays don't work well
    - We don't need these for screening but for filling gaps
    - Need to know what we are trying to model

Tool or assay

SWOT analysis: 3D tissue constructs

Threats

- **Validation of methods is very source intense**
- **Limited availability of tissue constructs, price, potential issues with patents (?)**
- **Variability of human tissues**

Seiichi Ishida NIHS, Japan

Tool or assay

SWOT analysis: Hepatotoxicity TEST by stem cell derived hepatocyte

Strengths

-Theoretically unlimited supply of human hepatocytes
-Supply of hepatocytes with different genetic background
-Supply of hepatocytes from patients suffering specific diseases
-Simultaneous analysis of multiple drug metabolism cascade in one cell
-Model human metabolism including polymorphisms (differences in susceptibilities)
-Surrogate for replacement, model human,
- **-Need to understand assay limitations**
- **-Need clarification on hazard ID (genetox, carcinogenesis)**
- **-Need to understand gene expression during differentiation**
- **-Sequence stem cell genome (cell banks can do this) – need to characterize epigenome as well**

Seiichi Ishida NIHS, Japan

Tool or assay

SWOT analysis: Hepatotoxicity TEST by stem cell derived hepatocyte

Weaknesses

- **Complexity of differentiation process of stem cells into hepatocytes**
- **Low reproducibility of differentiation process**
- **Expensive cost**

Seiichi Ishida NIHS, Japan

Tool or assay

SWOT analysis: Hepatotoxicity TEST by stem cell derived hepatocyte

Opportunities

-Replacement of human primary hepatocyte
-Replacement of animal toxicity testing
-Improvement of heptatotoxicity prediction
-Evaluation of hepatotoxicity, prediction of metabolites, prediction of induction of drug metabolism-relating enzymes, and prediction of drug-drug interaction
-Supply of metabolites to other testing, *i.e.* gentoxicity testing
-At early stage of drug development

Seiichi Ishida NIHS, Japan

Tool or assay

SWOT analysis: Hepatotoxicity TEST by stem cell derived hepatocyte

Threats

- **Complexity of differentiation process**
- **Requirements of higher hepatocyte activities**
- **Establishment of standards for hepatocyte qualification**
- **Hepatocyte progenitor cell line: HepaRG**

Tool or assay

SWOT analysis: Humanized Animal Models

Strengths

-Allows for better characterization of human hazard and risk potential- Human relevant

-Provides additional data on mode/mechanism of action

-Different knockout models (*p53*) – also crossed with *lacZ* animals; RIVM also has DNA repair-deficient models;

Tool or assay

SWOT analysis: Humanized Animal Models

Weaknesses

-Involves animal use
-Expensive (creation and maintenance)
-Low-throughput
-Transgenic models- human gene product in mouse environment

Tool or assay

SWOT analysis: Humanized Animal Models

Opportunities

-Allow for refinement in hazard and risk assessments-

- -decreased uncertainty
- -increased human relevance

-New technologies are decreasing the cost and expanding the model species

-Can be used to further define “toxicity pathways” thereby facilitating development of in vitro assays based on MoA

-Can be used to validate in vitro hypotheses

Tool or assay

SWOT analysis: Humanized Animal Models

Threats

- Models not widely available
- Models using different technologies may generate different results- may delay progress
- Lack of acceptance of this technology for advancing human health risk assessments

In vitro toxicity testing using hepatocytes

Strengths

- Theoretically unlimited supply of human hepatocytes
- Supply of hepatocytes with different genetic background
- Supply of hepatocytes from patients suffering specific diseases
- Simultaneous analysis of multiple drug metabolism cascade in one cell

In vitro toxicity testing using hepatocytes

Weaknesses

- Complexity of differentiation process of stem cells into hepatocytes
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In vitro toxicity testing using hepatocytes

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