

In vitro to *in vivo*:
a difficult climb

In vitro alternatives to carcinogenicity

The equation *mutation = cancer* is not of general validity

Out of > 100 STTs, only *Salmonella* and Cell Transformation (to be confirmed on larger database) predict chemical carcinogenicity

in vitro alternatives: state-of-the-art

- Toxicokinetic
- Acute toxicity *Reduction*
- Skin irritation and corrosion *Replacement*
- Skin sensitisation
- Eye irritation *Reduction*
- Acute systemic and local toxicity
- Genotoxicity *Reduction*
- Carcinogenicity *Reduction*
- Repeated dose toxicity
- Reproduction
- Developmental toxicity
- Ecotoxicity

“Existing approaches incorporating replacement, reduction and refinement of animal testing: applicability in food and feed risk assessment” *The EFSA Journal* (2009) 1052, 1-77

In spite of intensive use of genomics and proteomics technologies

Drug Innovation on the Decline

- ...The number of new drugs entering the U.S. market has declined sharply, while spending by the pharmaceutical industry on research and development has steadily increased, congressional investigators said in a recent report..... *D Young, [February 1, 2007, AJHP News]*
-Currently, the two single most important reasons for attrition in clinical development are (i) lack of efficacy and (ii) clinical safety or toxicology, which each account for 30% of failures.... *AL Hopkins, Nature Chemical Biology 4, 682 - 690 (2008)*

Prediction of Toxcast carcinogenicity through *in vitro*

- All representative assays (n=30) are used

Assays	sqcc	
	<u>Mouse</u>	<u>Rat</u>
30 representatives	0.04	0.09

- Toxcast *in vitro* assays are **poorly correlated** with animal carcinogenicity

Benigni, submitted

http://www.epa.gov/NCCT/toxcast/files/summit/ToxcastDataSummit_Poster_Benigni%20May2009.ppt

Toxcast: *in vitro* / *in vivo*

- Rat Cholinesterase Inhibition

versus

“Nervous system factors” Cluster

- Mouse Liver Necrosis

versus

“Signaling (cell cycle, apoptosis...)” Cluster

No correlation

Toxcast data confirm previous evidence from drug design (omics) and traditional *in vitro* alternatives

- *in vitro* / *in vivo* gap: still crucial scientific issue
- Systemic effects component necessary
- Replacement with stand-alone *in vitro* tests: far
- Room for Reduction:
e.g., omics to rationalize observations in tissues;
HTS patterns for specific chemical classes

Systemic effects

- Cancer is not a condition of isolated cells, but a condition of tissues
- e.g. gene expression profiles of non-tumor cells (adjacent to tumors) provide much better prognosis than tumor cells in individual patients
Kalluri and Zeisberg, Nature Revs.Cancer, 2006
- e.g. invasiveness of cancer mainly influenced by stromal cells characteristics
Hoshida et al., NEJM, Nov. 6, 2008
- **Microenvironment / tissue is crucial**

In vitro alternatives to carcinogenicity

The equation *mutation = cancer* is not of general validity

Out of > 100 STTs, only *Salmonella* and cell transformation (to be confirmed on larger database) predict chemical carcinogenicity

Why these *in vitro* are successful ?

- **Salmonella** detects a specific mechanism shared by a large class of carcinogens
- **Cell transformation** simulates general tumoral phenotypic features shared by many mechanisms / pathways

Going too deep into molecular mechanisms: a blind alley ?