PMI RESEARCH & DEVELOPMENT

Explorative Toxicological Assessment of Consumer Products

Rosemarie B. Lichtner
Philip Morris International Research and Development,
Philip Morris Research Laboratories, Cologne, Germany

Goal

- Developing products with the potential to reduce the risks of smoking-related diseases is one of our top priorities.
- We are working to understand the underlying mechanisms of the diseases caused by smoking.
- Philip Morris International is committed to the 3R principles – Replacement, Reduction, and Refinement – according to the “Policy on the Welfare of Laboratory Animals Used in Research”.
  http://www.forschung3r.ch/de/publications/bu7.html

The Explorative Toxicology Project: Definition

- Explorative Toxicological Assessment searches for mechanisms of harm induction by single tobacco smoke-derived compounds.

- These effects may need exposure to low, sub-toxic concentrations of compounds for extended periods of time

  → pathophysiological effects.

Project Objectives

- Identify the toxicological potency of CS-derived compounds
- Identify molecular targets in mechanism-driven and direct screening assays
- Generate high throughput large scale omics data with candidate compounds in selected cell lines for bioinformatics analysis
- Test candidate compounds in functional disease-relevant *in vitro* assays
- Generate high throughput large scale omics data in functional disease-relevant *in vitro* assays for bioinformatics analysis

Key Assumptions

- Acute toxic effects of compounds are of minor importance for disease
- Compounds cause measurable pathway perturbations at low, sub-toxic concentrations
- These pathway perturbations are relevant for mechanistic disease understanding
- Systems-response profiles of single compounds in conventional and organotypical cell cultures reflect at least partially the response profiles in living organisms/organs

The ToxCast Project

- National Academy of Sciences report (2007): Toxicity testing in the 21st Century
- The U.S. Environmental Protection Agency launches the ToxCast program in 2007
- Integration of molecular biology with chemistry and modern computing
- Mechanistically informative *in vitro* assays for screening methods
- Establishment of “toxicological pathways” providing systems level understanding of biological processes and their perturbation

Approach

- Select compounds from mainstream cigarette smoke (CS)
- Select cell culture assay systems known to respond to CS
- Compound screening from simple to complex assays: limiting the number of compounds for further testing
- Use systems biology (omics) to elucidate mechanisms of pathophysiology.

Well-known Smoke-derived Compound Classes

- Phenols and Polyphenols
- Polycyclic aromatic hydrocarbons
- Aldehydes
- Aromatic amines
- N-nitrosamines
- N-Heterocycles
- O-Heterocycles
- Alkaloids
- Cyclic isoprenoids
- Phytosterols
- Acids and Amides
- Heavy metals and inorganics

Leading from Simple to Complex Assays towards a Systems Approach

Cell-based analysis of proliferation/inhibition

Cell-based molecular target-specific assays

Selected cell lines: omics approaches

Complex cell cultures known to react to CS

Selected cell cultures: omics approaches

Normal Human Bronchial Epithelial Cells (NHBE)

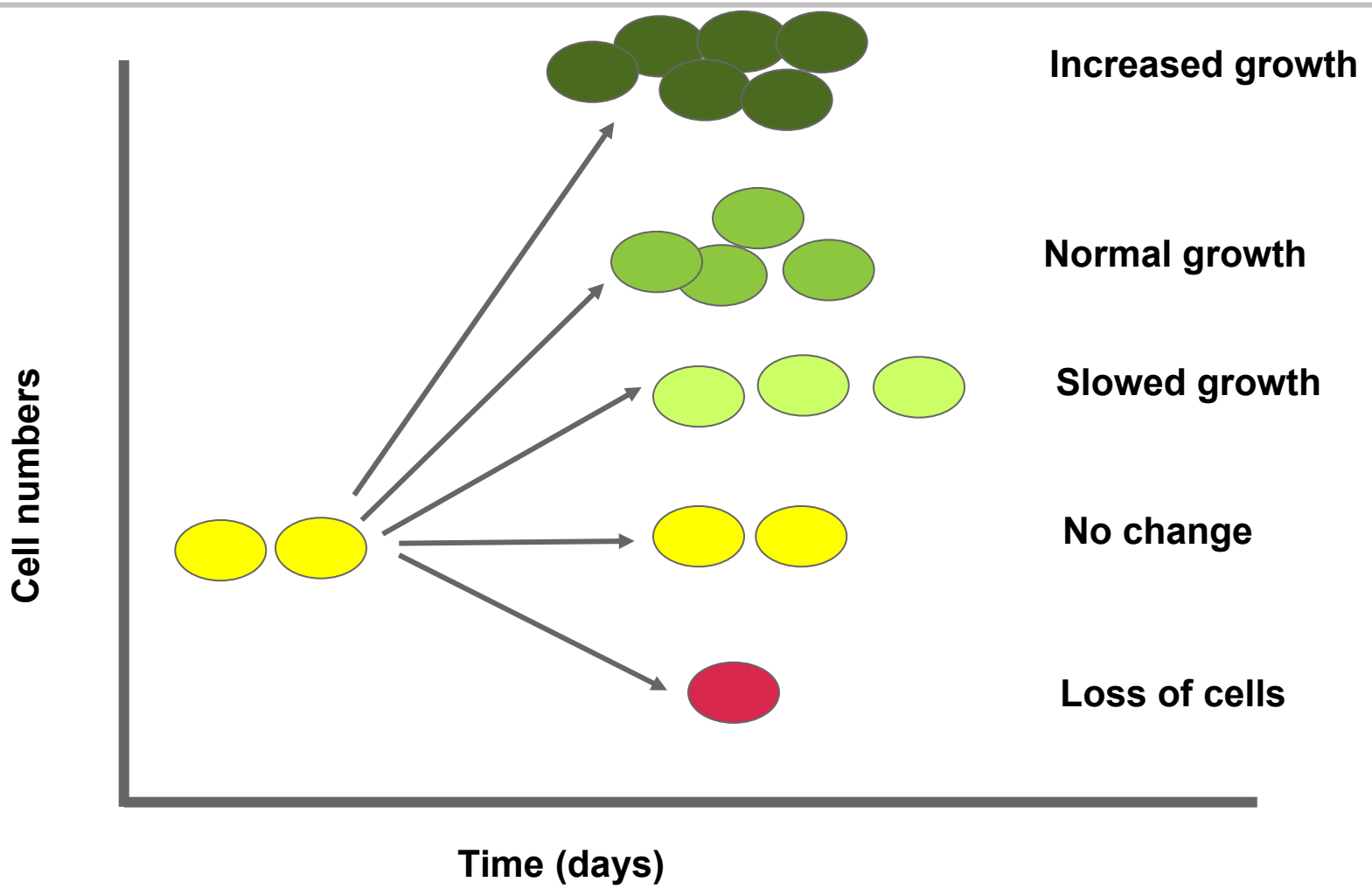
Inhaled smoke is initially in contact with quiescent lung epithelial cells. Upon lung injury, also proliferating epithelial cells will be present in the lung.

NBHE cells:

- Primary cell lines with limited life span
- Variability between donors
- Donor selection: Caucasian, non-smoker, adult

→ Quiescent and proliferating cells used for compound testing

Possible Effects of Compounds on Cell Growth



Decreased Biological Mass

Apoptosis can be triggered via dysfunction of mitochondria or by activation of specific cell membrane-bound death receptors

- Mitochondria dysfunction
- Overall apoptosis

No effects on Biological Mass

- Compound is biologically inert
- Compound has to be metabolically activated → Metabolic Activation
- Compound activates/inhibits target molecules not involved in cell proliferation/inhibition: Genotoxic compounds → Greenscreen assay
- Compound affects target molecule not present in cell line used (e.g., cell-specific transcription factors) → Reporter assays

Greenscreen Assay: Basic Principles

- Reporter assay with p53-dependent, *genotoxin specific* induction of human GADD45a expression
- Growth Arrest and DNA-Damage-inducible protein 45 alpha
- Role in cell cycle arrest, apoptosis, DNA repair, genomic stability
- Central role in the maintenance of genomic integrity
- Induced upon genotoxic stress
- Stimulates DNA excision repair in vitro and inhibits entry of cells into S phase

Gentronix

Reporter assays

Basic principle

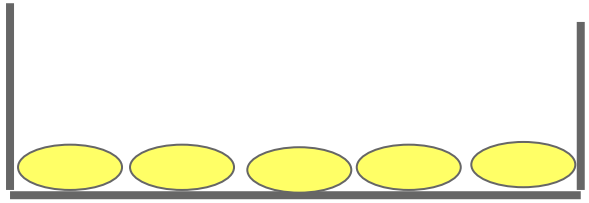
- Evaluation of transactivating potential of nuclear hormone receptors in cellular assays
- Test agonistic and antagonistic activity of smoke-derived compounds

Selected Cell Lines: omics Approaches

- The advantage of omics will be used to show effects of compounds at concentrations not yet resulting in overt altered phenotypes.
- The cell lines should: express the molecular target, be relevant for smoke-related diseases and non-transformed.
- The cells will be investigated using gene and protein expression (Affymetrix chips and reverse protein arrays).
- For compounds with putative genotoxic activity, NHBE cells will be used at longer exposure time points and subjected to analysis of gene copy alterations (CGH).

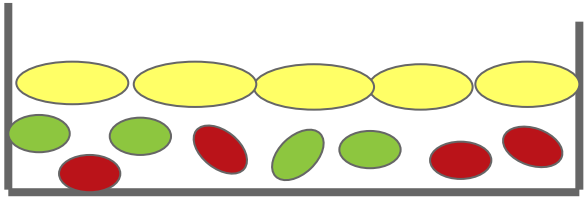
Complex Monolayer and Organotypical Cell Cultures

Complex monolayer and organotypical cell cultures known to react to CC



Monolayer or 2D culture

Cytokine induction
Gap-junctional intercellular communication
Epithelial permeability
Cell migration



Organotypical or 3D culture

Artificial airway epithelium
Artificial vessel wall

Summary

- The toxicological potency (hazard) of cigarette smoke-derived compounds will be estimated
- The molecular targets of selected compounds will be determined at low, sub-toxic concentrations
- High throughput large scale omics data will be generated in selected cell lines and functional assays with candidate compounds
- Bioinformatic analysis will be used to identify “toxicological pathways” in order to understand biological processes
- This insight will be used for the mechanistic understanding of (smoking-related) diseases

Acknowledgements

Co-authors

- Hans-Jörg Urban (Philip Morris Research Laboratories, Neuchàtel, Switzerland; up to February 2010)
- Katrin Stolle (Philip Morris Research Laboratories, Cologne, Germany)
- Walter K. Schlage (Philip Morris Research Laboratories, Cologne, Germany)
- Manuel C. Peitsch (Philip Morris Research Laboratories, Neuchàtel, Switzerland)

Special thanks also to all colleagues at Philip Morris Research Laboratories (Cologne, Germany) for providing excellent support.

Thank you for your attention