SIXTH FRAMEWORK PROGRAMME

IP PL 037712

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Non-genotoxic hepatocarcinogens specifically target mitochondria and gap junctions

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Introduction: Background

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The aim…

to develop omics-based *in vitro* screens for testing the carcinogenic potential of chemical compounds

REACH
(Registration, Evaluation, Authorization and Restriction of Chemicals)

30 000 chemicals need to be toxicologically tested e.g. carcinogenicity consumes high number of animals

Carcinogenicity

- Genotoxic carcinogens → A battery of *in vitro* and *in vivo* tests
- Non-Genotoxic carcinogens → No validated *in vitro* tests exist for examining the non-genotoxic carcinogenic potential of chemicals !!!

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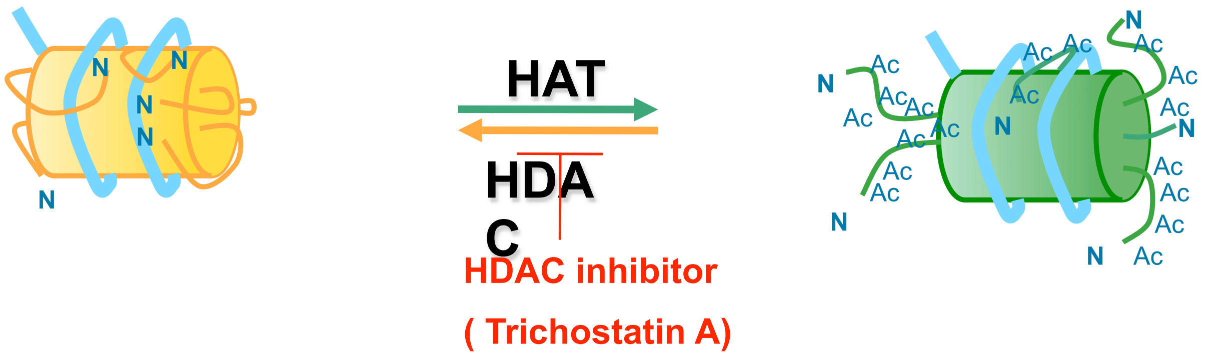
Introduction: Liver as a target

Suitable model: primary hepatocytes reflect the in vivo situation

Problem: cell cycle re-entry associated with dedifferentiation and loss of functionality

New strategy: primary hepatocytes + histone deacetylase inhibitors (HDACi) → **Stabilization**

Introduction: Mechanism of HDACi



Primary hepatocyte cultures treated with HDACi →

- -Improved differentiated state
- -Reduced apoptosis
- -Cell cycle arrest

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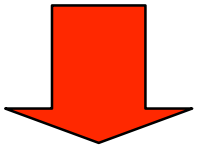
Aim

Question: Can stabilized hepatocytes be applied to screen for non-genotoxic/genotoxic carcinogens?

Rationale: Genotoxic substances target nucleus and cause a multitude of changes whereas non-genotoxic carcinogens target individual subcellular organelles e.g. mitochondria, gap junction intercellular communication (GJIC).



In vitro?

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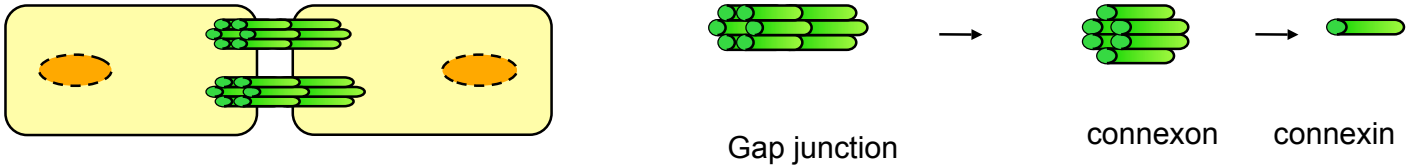
Endpoints

GJIC

- Key regulators of tissue homeostasis
- Cx32 most abundant connexin species in the liver
- Considered to be a differentiation marker
- Putative biomarker for detection of non-genotoxic carcinogens(in vivo data)



Mitochondria

- "Energy factory" of cells and tissues
- Mitochondrial activity: MTT test

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Training set

Non-genotoxic carcinogens	Genotoxic carcinogens
-Methapyrilene hydrochloride	-Benzo[a]pyrene
-Phenobarbital sodium	- 2-Nitrofluorene

Vinken et al., 2008,The carcinoGENOMICS project: Critical selection of model compounds for the development of omic-based in vitro carcinogenicity screening assays, Research Mutation 659, 202-210

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Results: Cx32

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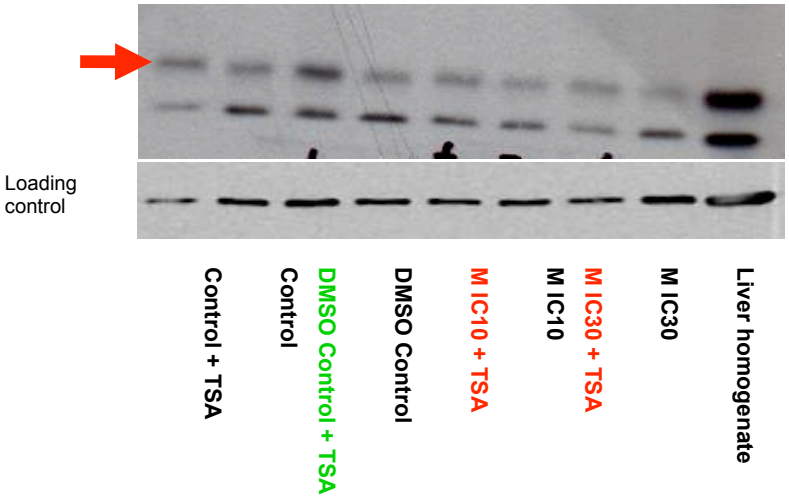
Methapyrilene. HCl: non-genotoxic



Downregulation of Cx32 confirms in vivo data

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Results: Cx32

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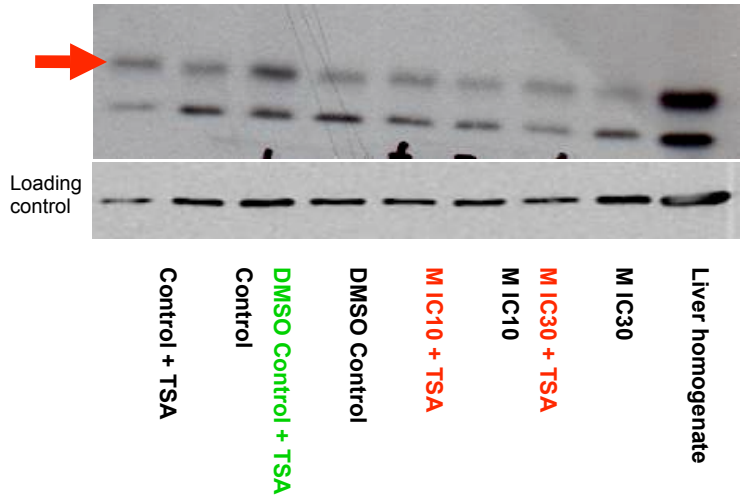
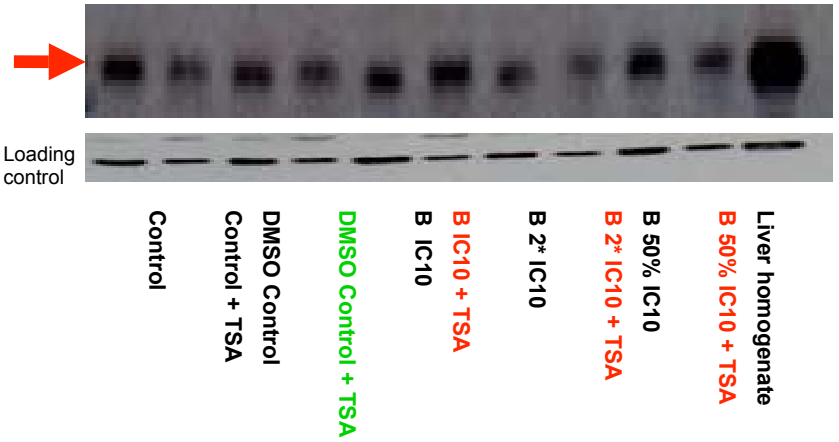
Methapyrilene.HCl: non-genotoxic



Downregulation of Cx32 confirms in vivo data

Benzo[a]pyrene: genotoxic



In contrast: No Cx32 downregulation Even slight increase

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Results: MTT

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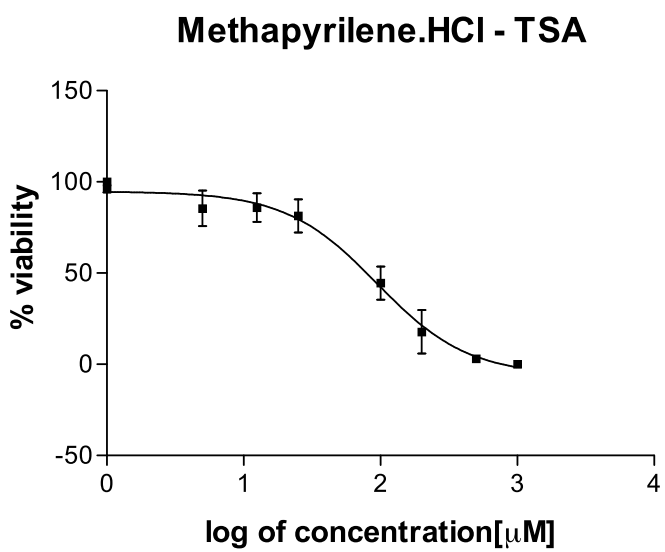
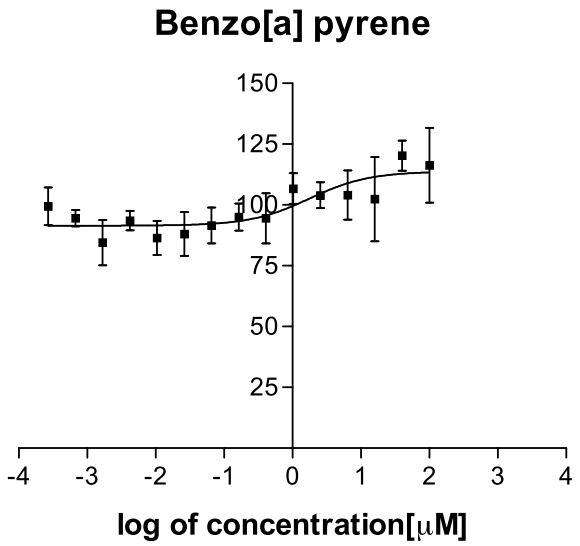
Methapyrilene.HCl: non-genotoxic



Dose dependent decrease of mitochondrial activity

Benzo[a]pyrene: genotoxic



No effect

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Results: MTT

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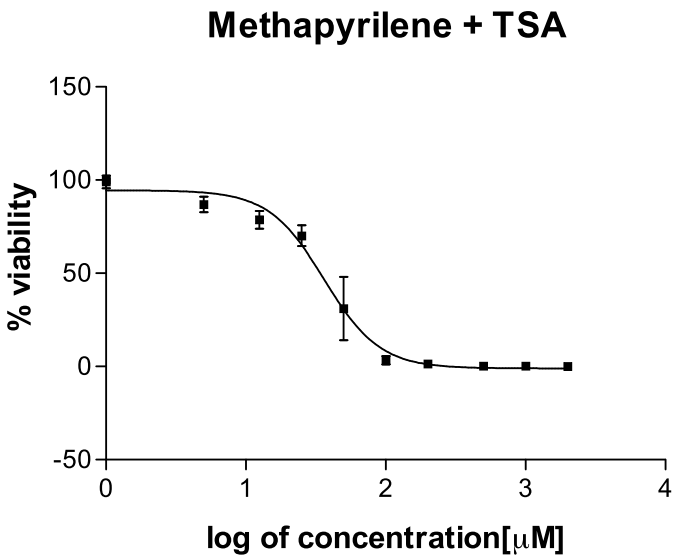
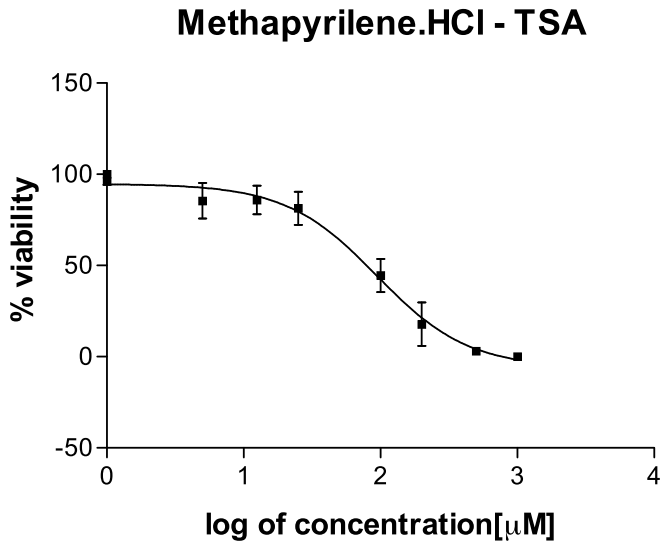
Methapyrilene. HCl: non-genotoxic





IC_{50}= 36.34 µM

IC_{10}= 3.65 µM

IC_{50}= 94.03 µM

IC_{10}= 9.03 µM

+ TSA = more sensitive system

Other results: 2-Nitrofluorene, Phenobarbital sodium, Diclofenac sodium

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Conclusions

- GJIC and mitochondrial activity: suitable *in vitro* markers for discriminating between genotoxic and non-genotoxic hepatocarcinogens

- TSA stabilized hepatocytes represent a more sensitive testing system

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Thank you for your attention!